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HEPATIC NECROSIS INDUCED BY DIETARY MEANS

I. Structural Changes Occurring in the Liver During the Development of Necrosis

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DESPITE the volume of investigative work which has been carried out in relation to the massive hepatic necrosis produced by dietary means, the mechanism of this process still remains to be fully elucidated. Many attempts have been made to arrive at an understanding of the etiologic factors involved, but no comprehensive study of the progressive development of this lesion of the liver has yet been published. In view of these considerations it seemed worth while to carry out a detailed investigation to ascertain the progressive structural and biochemical changes which occur prior to and at the time of development of the hepatic damage.

The original observation of the production of dietary hepatic necrosis was made by Weichselbaum [1] in 1935, but it seems clear from his paper that he was not aware of the exact nature of the lesion. He found that a high percentage of rats which were restricted to diets low in protein and deficient in cystine for approximately 6 weeks became sick and died. The livers were described as being swollen and hemorrhagic, and presumably were necrotic, although no histologic sections were described. Animals which received supplements of methionine or cystine remained healthy. In 1939 and later in 1942 György and Goldblatt [2] described fatty infiltration, necrosis and cirrhosis of the livers of rats which had been fed diets low in casein. The diets used were deficient both in lipotropic factors and in the sulfur-containing amino acids, methionine and cystine. Initially the authors attributed the hepatic damage to a deficiency of the "vitamin B_2 complex," but later considered the lesions

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1. Weichselbaum, T. E.: Quart. J. Exper. Physiol. **25**:363, 1935.

2. György, P., and Goldblatt, H.: (*a*) J. Exper. Med. **70**:185, 1939; (*b*) ibid. **75**:355, 1942.

to be a result of a dietary deficiency of lipotropic factors.[2b] They regarded necrosis and hemorrhage merely as a phase in the development of cirrhosis. The addition of casein, methionine, or cystine plus choline exerted a prophylactic effect against hepatic injury. It may be noted that a supplement of 20 mg. of choline per rat per day prevented the development of cirrhosis but not necrosis. The addition of 40 mg. of methionine daily completely inhibited the development of both cirrhosis and necrosis, but added cystine prevented only necrosis. Several other investigators also have reported that cirrhosis and necrosis were produced in animals by diets low in protein (Webster[3]; Earle and Victor[4]; Handler and Dubin[5]).

Daft, Sebrell and Lillie[6] first postulated that the necrosis and the cirrhosis described by György and Goldblatt[2] were separate and distinct entities, a conclusion which could be inferred also from the work of the latter investigators. Daft, Sebrell and Lillie found that choline had no influence on the development of necrosis and hemorrhage whereas both the sulfur-containing amino acids, methionine and cystine, when given in sufficient amounts prevented these lesions. Methionine or choline in adequate quantities prevented fatty infiltration and cirrhosis.

Himsworth and Glynn[7] presented evidence confirming the work and theories of Daft, Sebrell and Lillie. They employed a series of diets containing different percentages of casein and/or yeast as the sole source of protein and succeeded in producing hepatic necrosis independent of diffuse fibrosis. They found no evidence at necropsy to suggest infection as an etiologic agent. Carefully collected specimens of liver tissue cultured for bacteria and viruses gave negative results.

Unknown to American and English workers, Hock and Fink,[8] in Germany, were carrying out experiments of a similar nature. Rats were fed diets in which the protein moiety was supplied by various yeasts differing in their content of sulfur-containing amino acids. They demonstrated that the incidence and the severity of hepatic necrosis increased as the protein sulfur in the diet diminished. Cystine added to the diet at a level of 0.2 per cent prevented the hepatic damage whereas small but unspecified amounts of ascorbic acid failed to do so. They concluded that diets lacking or deficient in cystine resulted in a severe metabolic dis-

3. Webster, G. T.: J. Clin. Investigation **21**:385, 1942.

4. Earle, D. P., and Victor, J.: J. Exper. Med. **75**:179, 1942.

5. Handler, P., and Dubin, I. N.: J. Nutrition **31**:141, 1946.

6. Daft, F. S.; Sebrell, W. H., and Lillie, R. D.: Proc. Soc. Exper. Biol. & Med. **50**:1, 1942.

7. Himsworth, H. P., and Glynn, L. E.: Clin. Sc. **5**:93 and 133, 1944; Proc. Roy. Soc. Med. **38**:101, 1944; Lancet **1**:457, 1944.

8. Hock, A., and Fink, H.: Ztschr. f. physiol. Chem. **278**:138, 1943; **279**:187, 1943.

turbance leading to hepatic necrosis. The authors suggested that cystine may be required for the breakdown of the end products of metabolism and that in its absence these substances act as cellular toxins.

Glynn, Himsworth and Neuberger[9] designed a series of diets utilizing purified amino acids as the sole source of protein. The animals that received diets devoid of cystine and low in methionine (8 mg. per rat per day) died of hepatic necrosis in 30 to 55 days. The rats which received diets high in methionine (80 mg. per rat per day) or in cystine (64 mg. per rat per day) lived for more than 100 days and showed no evidence of hepatic injury. Since the sulfur moiety of methionine can be utilized by the body in the synthesis of cystine (Tarver and Schmidt[10]), the authors considered the development of hepatic necrosis to be due specifically to a deficiency of the latter amino acid.

Other competent workers,[11] utilizing diets similarly deficient in protein and in the sulfur-containing amino acids, failed to obtain comparable results. György and Goldblatt,[12] in discussing their work on dietary hepatic injury, stated: ". . . we were unable to produce necrosis at will and in this respect we have not made any substantial progress beyond our first observations . . ." made in 1939.[12a]

An examination of the diets used by authors reporting a significant incidence of necrosis revealed several interesting points of similarity which were lacking in the diets of those who failed to produce the hepatic lesion. All diets reviewed by us which were notably successful in producing necrosis of the liver contained cod liver oil as a source of vitamins A and D; the fat component was relatively low in tocopherols and, furthermore, alpha tocopherol was not included in the vitamin supplements. These points were taken into consideration in the formulation of diets for the production of hepatic necrosis. A high incidence of necrosis has been obtained on such diets (Abell and Beveridge[13]).

It may be pointed out that the prophylactic role of alpha tocopherol in hepatic necrosis has been established recently in publications by Himsworth and Lindan[14] and by György and Goldblatt.[12] Of interest in

9. Glynn, L. E.; Himsworth, H. P., and Neuberger, A.: Brit. J. Exper. Path. **26**:326, 1945.

10. Tarver, H., and Schmidt, C. L. A.: J. Biol. Chem. **130**:67, 1939.

11. Irving, J. T., and Schwarz, H. M.: Clin. Proc. **5**:14, 1946. Neuberger, A., and Webster, T. A.: Biochem. J. **41**:449, 1947. Sellers, E. A.; Lucas, C. C., and Best, C. H.: Unpublished data, cited by Sellers, E. A.: Canad. M. A. J. **59**:403, 1948. Radhakrishna Rao, M. V.: Nature, London **161**:446, 1948. Hall, C. A., and Drill, V. A.: Proc. Soc. Exper. Biol. & Med. **70**:202, 1949.

12. György, P., and Goldblatt, H.: J. Exper. Med. **89**:245, 1949.

13. Abell, M. R., and Beveridge, J. M. R.: Canad. J. Research, Sect. E **27**:316, 1949.

14. Himsworth, H. P., and Lindan, O.: Nature, London **163**:30, 1949.

this respect is the work reported in 1944 by Schwarz,[15] who produced extensive necrosis of the liver in rats by utilizing an alkali-treated casein as a source of protein and demonstrated that alpha tocopherol prevented the lesion.

After it had been demonstrated that necrosis could be consistently produced by dietary means, the experiment to be described was carried out to ascertain the progressive structural and biochemical changes which occur prior to and at the time of development of massive hepatic necrosis.

EXPERIMENTAL PROCEDURE

A total of 121 male rats, average weight 141 Gm. (102 to 183 Gm.), were housed in individual wire cages with ½ inch (8 mm.) mesh screen floors. Eighty-one of the animals were of the Wistar strain, and the remaining 40 were hooded rats.

Composition of Diet

Yeast (Fleischmann's active dried bakers' yeast)*	18
Corn starch	71
Lard	5
Salt mixture†	3
Cod liver oil (Mead Johnson & Company, 3,000 international units of vitamin A and 400 international units of vitamin D per gram)	2
Sugar-vitamin mixture (explained in text)	1

* The yeast was ground to pass a no. 20 mesh screen (courtesy of Charles R. Will & Company, London, Ont.)

† Sodium chloride, 22 Gm.; calcium phosphate (dibasic), 130 Gm.; potassium citrate, 129 Gm.; magnesium sulfate (crystalline), 30 Gm.; iron citrate, 5 Gm.; trace mixture, 0.7 Gm. The trace mixture was composed of the following salts: potassium iodide, 13 Gm.; sodium fluoride, 10 Gm.; manganese sulfate (anhydrous), 2 Gm.; cupreous iodide, 1 Gm.; aluminum potassium sulfate (anhydrous), 1 Gm.; zinc sulfate, 1 Gm. Hilmsworth used the salt mixture for experiments published in 1944 (see footnote 7 of this paper); since the composition was not available in the literature he forwarded the information to us.

Since no differences were observed in the changes produced in the two strains of rats by the dietary regimen used, they were treated as a homogeneous group. Eight grams of the test diet (table) were offered daily, and water was allowed ad libitum. A weekly record of changes of body weight was made.

The diet was prepared at approximately weekly intervals and stored in tightly closed cans at 0 — 10 C. The sugar-vitamin mixture supplied the following amounts of B vitamins per 8 Gm. of food: thiamine hydrochloride, 0.02 mg.; riboflavin, 0.025 mg.; pyridoxine hydrochloride, 0.02 mg., and calcium pantothenate, 0.1 mg.

The animals were fed fox chow ad libitum for a period of 5 days. At the end of this time, 5 animals, serving as normal controls, were killed after a fasting period of 10 hours. The remaining rats were given the experimental diet. Groups of 5 animals selected at random were killed similarly during the experimental period on the 15th, 29th, 36th, 43d, 51st, 58th, 61st, 64th, 67th, 71st, 74th, 78th, 81st, 85th, 88th days and the remaining 6 animals on the 92d day. It may be noted that from the 48th day on, some of the animals died of massive necrosis or were killed at the point of death.

The animals selected for killing and those that were moribund when killed were examined immediately, and the following routine was performed. Small wedges of tissue were taken from the three main lobes of the liver and plunged immediately into tared tubes containing 30 per cent (W/V) potassium hydroxide solution.

15. Schwarz, K. C.: Ztschr. f. physiol. Chem. **281**:101, 1944; **281**:109, 1944.

These were used for estimations of glycogen. The livers were then weighed, and thin slices of tissue were taken from each lobe and fixed in Zenker-formaldehyde solution, formaldehyde solution U. S. P. diluted 1:10 and a modified Carnoy's solution (10 per cent formaldehyde solution U. S. P. and 5 per cent glacial acetic acid in absolute alcohol, V/V). The remaining portions of the livers (2 to 4 Gm.) were used for the determination of certain lipid fractions (Abell and Beveridge [16]).

Animals that died during the course of the experiment were submitted to autopsy as soon as possible, and the livers were dealt with as described in the foregoing paragraph except that tissue was not taken for glycogen estimations or stains.

Sections of all livers were stained with hematoxylin (Ehrlich's) and eosin, and with Masson's trichrome stain. Glycogen stains were carried out on tissue from the livers of all rats which were killed, according to the method described in Lillie's "Histopathologic Technique," [17] using Best's carmine stain. The method of Krajian [18] utilizing scarlet red was followed in the preparation of fat stains on frozen sections from tissue fixed in formaldehyde solution.

RESULTS

At the end of 15 days there was a marked loss of body weight (average, 20 Gm.), after which little change was observed. The general appearance of the animals remained good until the development of necrosis.

Eight animals died of acute intercurrent infections during the experiment; 2 animals during the first 3 weeks, 4 with pneumonia after 52 to 54 days, and the remaining 2 after 82 to 83 days. One of the latter animals showed a localized area of acute hepatic necrosis histologically similar to the necrosis produced by dietary means. Several of the others showed areas of focal necrosis due to an associated paratyphoid infection. Although this experiment was not drawn up to determine the incidence of necrosis induced by the diet used, it is interesting that a total of 45 animals presented evidence of hepatic necrosis attributable to deficiencies in the diet. Undoubtedly necrosis would have developed in many more had they not been put to death during the early part of the experimental period. The same diet has been shown to produce necrosis in 19 of a group of 20 rats in an experiment terminated at 105 days.

Structural Changes Prior to the Development of Necrosis.—The liver lobules of the control animals presented a normal, orderly appearance. The cords and cells were well defined. The cytoplasm had a somewhat reticulated appearance indicating that a normal amount of glycogen had been present in the tissue prior to fixation. A prominent feature was the presence of numerous well defined basophilic granules,

16. Abell, M. R., and Beveridge, J. M. R.: Arch. Path., this issue, p. 23.

17. Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948.

18. Krajian, A. A.: Histological Technic, St. Louis, C. V. Mosby Company, 1940.

the so-called protein storage particles of Berg. These are now thought to be ribonucleic acid aggregates[19] (fig. 1 *A*). The nuclei were clearly outlined and regular in appearance. Frozen section stained with scarlet

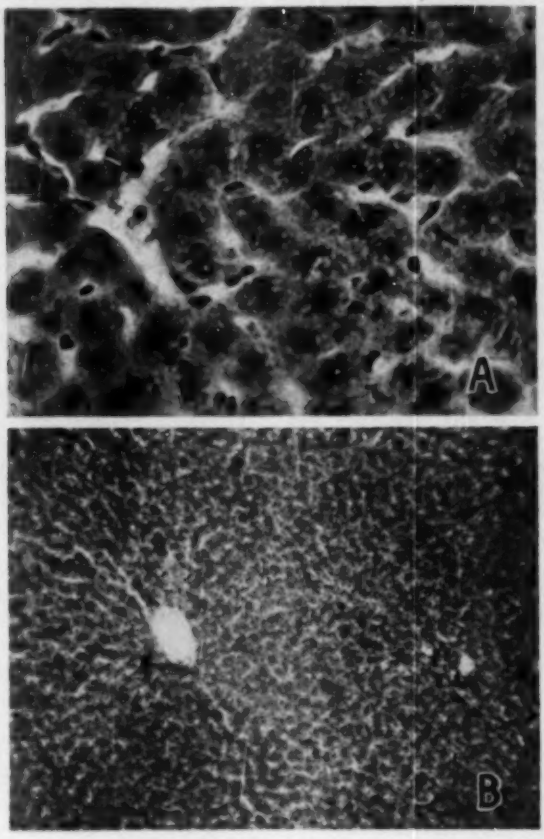

Fig. 1.—*A*, section of the liver of a normal control rat. The cells about the central vein contain an abundance of cytoplasm and numerous basophilic granules. Hematoxylin and eosin; × 440. *B*, section of the liver of a normal control rat. There is no increase of stainable lipid in the cells about the central vein. Frozen section stained with scarlet red; × 90.

19. (*a*) Kosterlitz, H. W., and Campbell, R. M.: Nutrition Abstr. & Rev. **15**:1, 1945. (*b*) Kosterlitz, H. W.: J. Physiol. **106**:194, 1947.

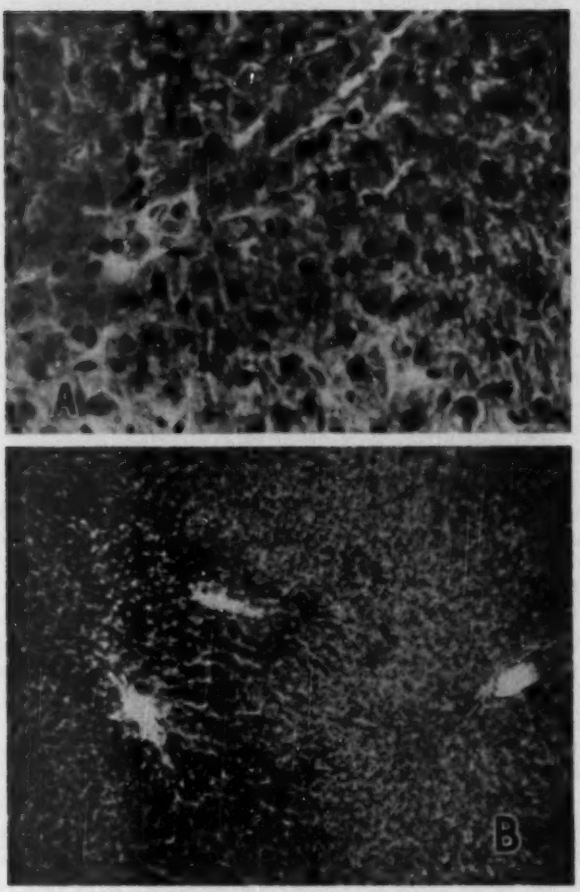

Fig. 2.—*A*, section of the liver of a rat which was killed after having been restricted to the experimental diet for 15 days. The central liver cells are smaller, and the basophilic granules are decreased in number and not so well defined. Hematoxylin and eosin; × 440. *B*, section of the liver of a rat killed after 15 days of the diet. There is a prominent increase of stainable lipid in the cells about the central veins. Frozen section stained with scarlet red; × 90.

red showed essentially no stainable lipids except an occasional globule in the peripheral zone (fig. 1 *B*).

The livers of rats killed after 15 days on the test diet revealed a definite decrease in size of the hepatic lobules. The cells were smaller because of a loss of cytoplasm, and the nuclei were more closely approximated. The basophilic granules were not so numerous nor so well defined as in the control livers (fig. 2 *A*). Glycogen stains revealed no appreciable change. Frozen sections stained with scarlet red demonstrated fine droplets of stainable fat in the cells about the central vein, producing a definite halo effect (fig. 2 *B*).

Livers of animals killed on the twenty-ninth day presented findings somewhat similar to those in rats killed on the fifteenth day. The basophilic granules, however, were not quite so prominent. The glycogen content of the cells was similar, but in some lobules the granules were more prominent in the cells about the portal tract. Fat stains revealed a further increase of stainable lipid in the cells around the central veins (fig. 3 *A*), and in the hematoxylin and eosin sections these cells presented a fine fatty vacuolation or a honeycomb appearance.

The only consistent change which was observed in the remaining groups of animals that were killed prior to the development of necrosis was a progressive increase in the lipid content of the central liver cells (figs. 3 *B* and 4). This fatty appearance of the cells, which was readily recognized in the hematoxylin and eosin sections and confirmed by fat stains, was most marked in the left lobes. Occasionally all the cells within the lobules had a foamy appearance. Glycogen granules were usually more prominent in the cells about the portal areas.

The livers displaying evidence of necrosis were divided on the basis of the gross and microscopic observations into three distinct groups—(1) massive necrosis, (2) submassive and recurrent submassive necrosis and (3) postnecrotic scarring.

It may be noted at this point that the microscopic appearance of the sections of all necrotic livers stained with scarlet red was so variable that no generally applicable conclusions could be drawn from them.

Massive Hepatic Necrosis.—Eleven animals died of massive necrosis, and 9 animals that were killed when moribund presented the same lesion. Death due to massive necrosis occurred at an average of 71 days (48 to 88) on the test diet.

The animals in which this lesion developed appeared healthy and active until a period ranging from 12 hours to 3 days prior to death. Frequently they seemed perfectly normal and consumed their full allotment of food on the day preceding death. The first manifestations of illness were a listless, dumpy appearance, ruffling of the fur and a yellowish orange staining around the urethral meatus. The condition of

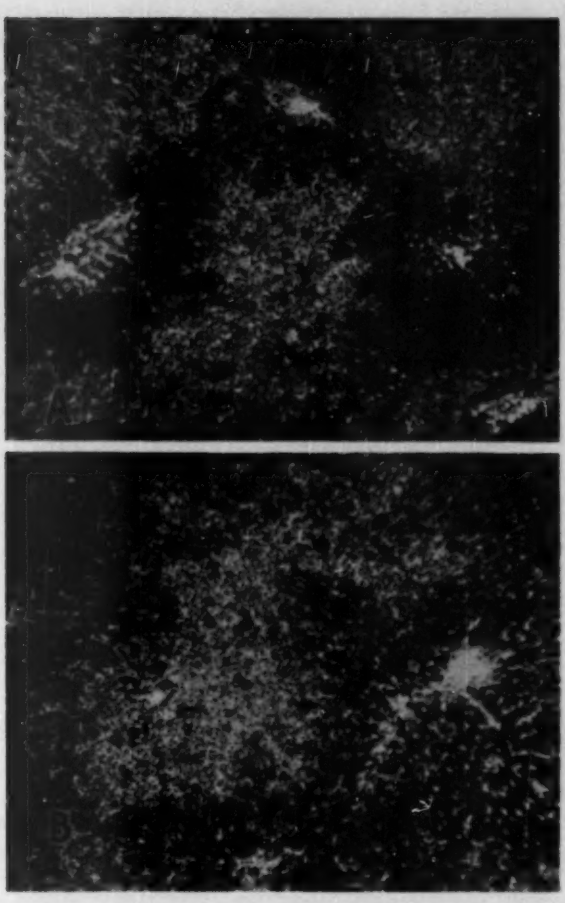

Fig. 3.—*A*, section of the liver of a rat which was killed after it had been fed the experimental diet for 29 days. There is a further increase of stainable lipid as compared with figure 2*B*. *B*, section of the liver of a rat killed after 43 days of the diet. There is a further increase of lipid material. Frozen sections stained with scarlet red; × 90.

the rats deteriorated rapidly, with a fall in body temperature followed by coma and death, occasionally with convulsions.

At autopsy, the livers were enlarged (see part II, which follows), soft, friable, with tense smooth capsules, and most of them presented a

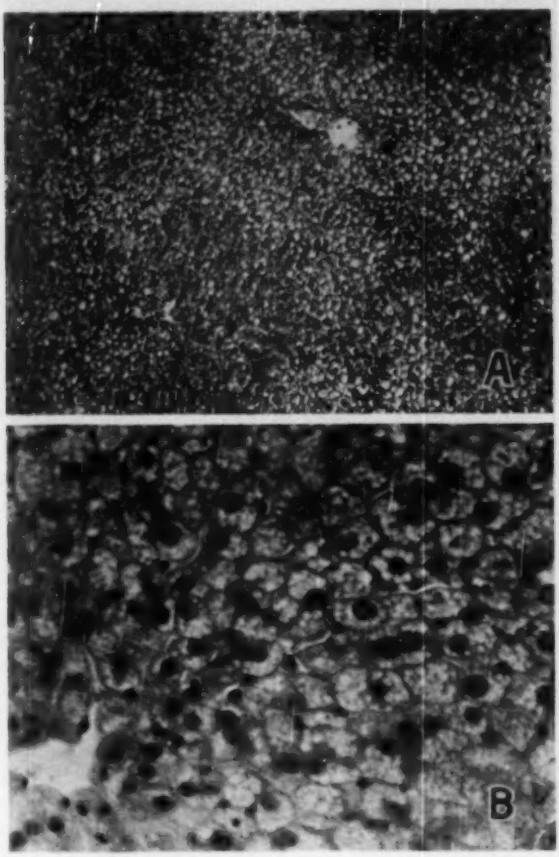

Fig. 4.—*A*, section of the liver depicted in figure *3B*. Hematoxylin and eosin; × 90. *B*, section of the liver depicted in figure *3B* and in *A* of this figure, showing fine fatty vacuolation or a honeycomb appearance of the cells about a central vein. Hematoxylin and eosin; × 440.

coarsely mottled appearance due to yellowish gray necrotic and hemorrhagic areas (fig. 5).

On section the livers displayed the same mottled appearance and were quite edematous. All lobes showed gross involvement, but hemorrhage was often more prominent in the left lobe, and this lobe also appeared to be more edematous. The livers of a few animals, however, merely presented the appearance of a frank chronic passive congestion.

Microscopically, the necrosis was most extensive, involving completely many contiguous lobules and the central and midzones of many other lobules (fig. 6). In the areas where viable liver cells remained,

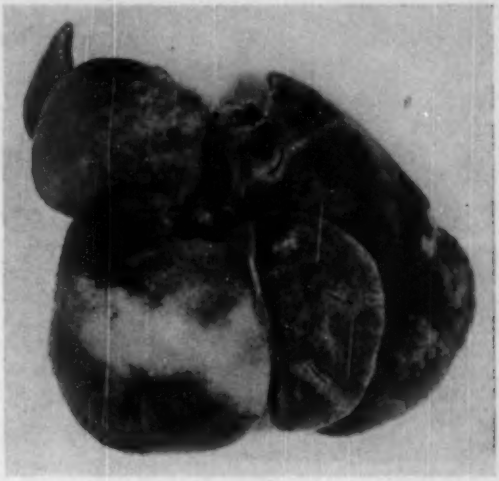

Fig. 5.—Massive hepatic necrosis. All lobes show gross involvement, but hemorrhage is more prominent in the left lobe.

these were situated near or around the portal tracts. These islands of living cells bordered sharply on the necrotic areas, and from some of them columns of surviving cells projected, finger-like, into the surrounding necrotic tissue.

The necrosis was coagulative in type, and in the early stages the liver cells were pale, finely granular, with distinct cell outlines, and showed striking nuclear changes consisting of pyknosis, karyolysis and karyorrhexis (fig. 7). At this stage there was no inflammatory cell infiltration, no hemorrhage and only slight congestion of the sinusoids. The blood vessels and the bile ducts appeared to be normal. Later the necrotic areas were more disorganized, consisting of pale eosinophilic debris, in which there were scattered nuclear fragments, a few neutro-

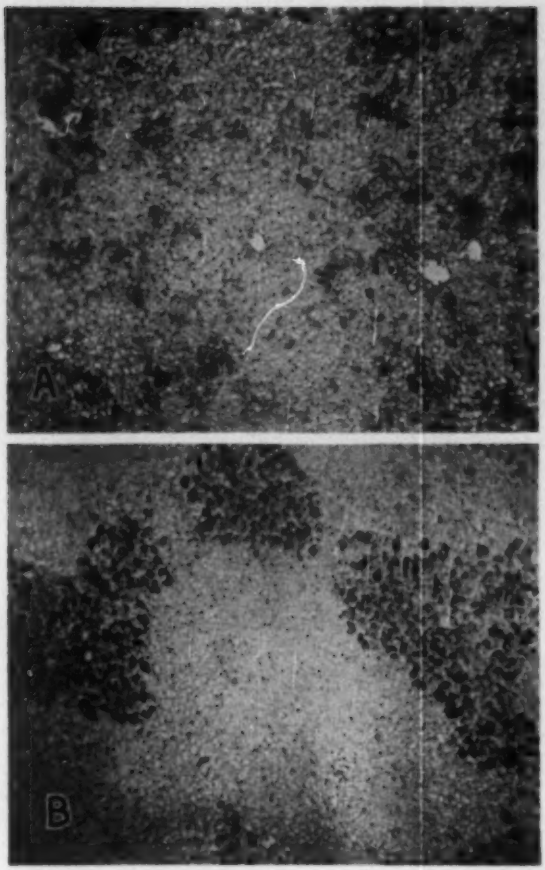

Fig. 6.—*A*, section of a rat's liver showing massive necrosis. There is complete lobular destruction with no surviving liver cells. The darker areas represent collections of erythrocytes. Hematoxylin and eosin; × 90. *B*, section of rat's liver showing massive necrosis. There is a complete absence of glycogen in the necrotic cells whereas the surviving cells about the portal areas contain normal amounts. Best's carmine stain; × 90.

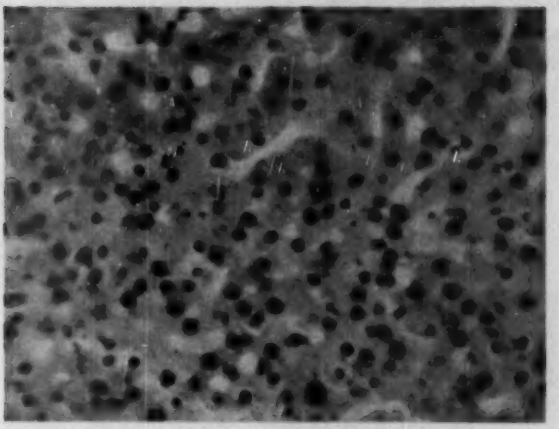

Fig. 7.—Section of rat's liver showing karyolysis and karyorrhexis as seen in an early phase of massive necrosis. Hematoxylin and eosin; × 400.

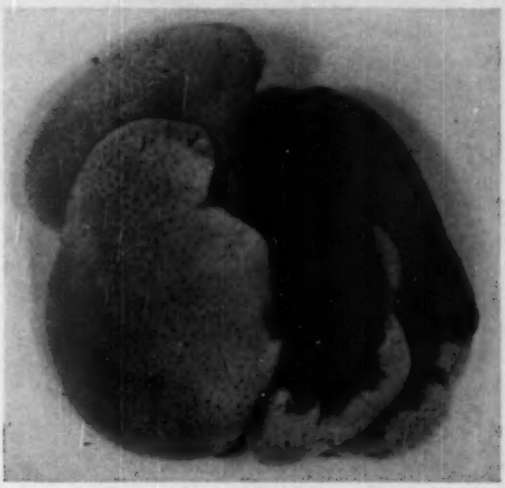

Fig. 8.—Submassive necrosis. The lesion is limited to the left lobe and the left half of the median lobe. This liver was from an animal killed after random selection.

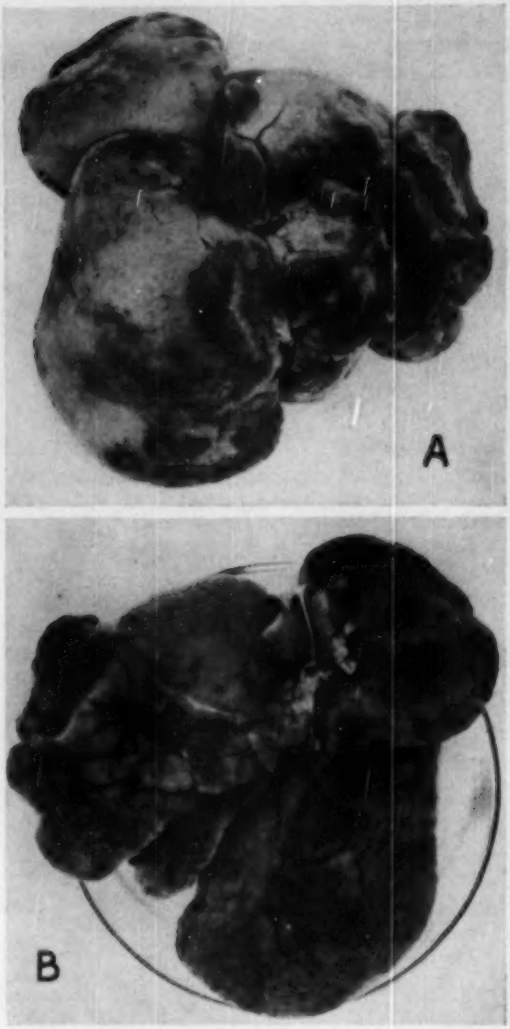

Fig. 9.—*A*, recurrent submassive necrosis. There are hemorrhage, necrosis, nodular areas of regeneration, and irregular, depressed scars. The latter features are more prominent over the left half of the liver. *B*, recurrent submassive necrosis. The under surface of the liver depicted in *A* is shown. The left lobe shows the greatest degree of deformity.

philic and eosinophilic cells and variable numbers of red blood cells indicating recent hemorrhage. The massive destruction of liver tissue had apparently occurred suddenly, and there was no evidence of regeneration of liver cells, no increase in the number of macrophages and no hemosiderin pigment.

Best's carmine stains carried out on liver sections from killed animals showing massive necrosis revealed that glycogen had been completely lost from the necrotic cells but that a moderate amount remained in the surviving cells about the portal areas (fig. 6*B*).

Submassive and Recurrent Submassive Necrosis.—Two animals which were killed after random selection and one animal which died of

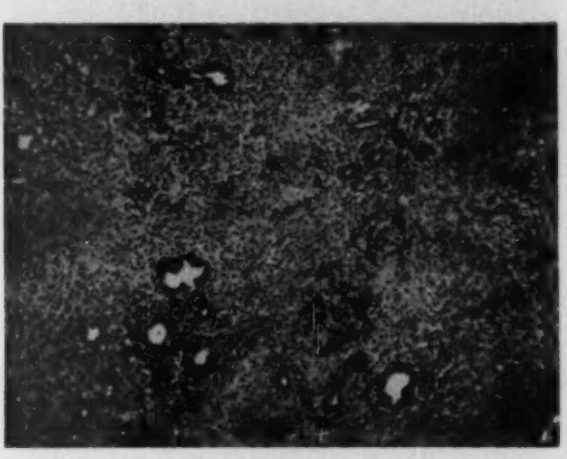

Fig. 10.—Section of a rat's liver showing recurrent submassive necrosis. In the center of the field is an area of previous necrosis with early fibrosis and regeneration of cells at the periphery. Bile ducts appear to be increased in number. Hematoxylin and eosin; × 90.

an intercurrent infection presented the findings of a single episode of submassive necrosis. The lesion was histologically similar to the lobular necrosis seen in massive involvement but was limited to the left half of the liver (the left lobe and the left half of the median lobe, fig. 8).

Recurrent bouts of submassive necrosis were observed in 14 animals, of which 1 died, the remainder having been killed when near death or selected at random for autopsy. The livers presented a variform appearance with nodular areas of yellowish regenerating tissue, bright red areas of recent necrosis and hemorrhage, faded reddish brown, slightly depres-

sed areas indicative of old necrosis and hemorrhage, and scattered depressed scars causing shrinkage and deformity of the lobes involved (fig. 9). The livers were usually normal in size or slightly enlarged, depending on the amount of tissue involved in the acute necrotic process. Fibrosis was more prominent in the left lobe and in the left half of the middle lobe.

The microscopic features comprised areas of acute necrosis of varying stages, recent and old hemorrhage, prominent areas of regeneration, numerous macrophages and areas of fibrosis. The necrosis, although submassive in extent, was similar to that seen in massive necrosis with

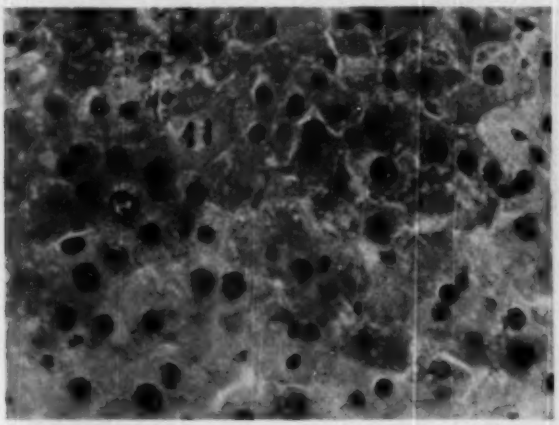

Fig. 11.—Section of an area of regeneration from the liver of a rat which showed recurrent submassive necrosis. Mitotic figures are numerous. Hematoxylin and eosin; × 440.

varying degrees of central, midzonal and complete lobular involvement. In some areas the necrosis appeared to be recent, merely producing prominent nuclear changes, whereas in others it appeared as amorphous masses of eosinophilic debris, indicative of a process of longer duration. Loosely arranged scars were scattered throughout the liver and consisted of cellular debris, plump young fibroblasts and numerous macrophages, many of which contained hemosiderin. Other scars consisted of dense fibrous tissue. The bile ducts and canaliculi were increased in number, but there was no evidence of obstruction (fig. 10).

Regeneration of cells (fig. 11) from the portal areas was a prominent feature, characterized by numerous large atypical cells with large nuclei and prominent nucleoli, frequent binuclear and occasional multinuclear

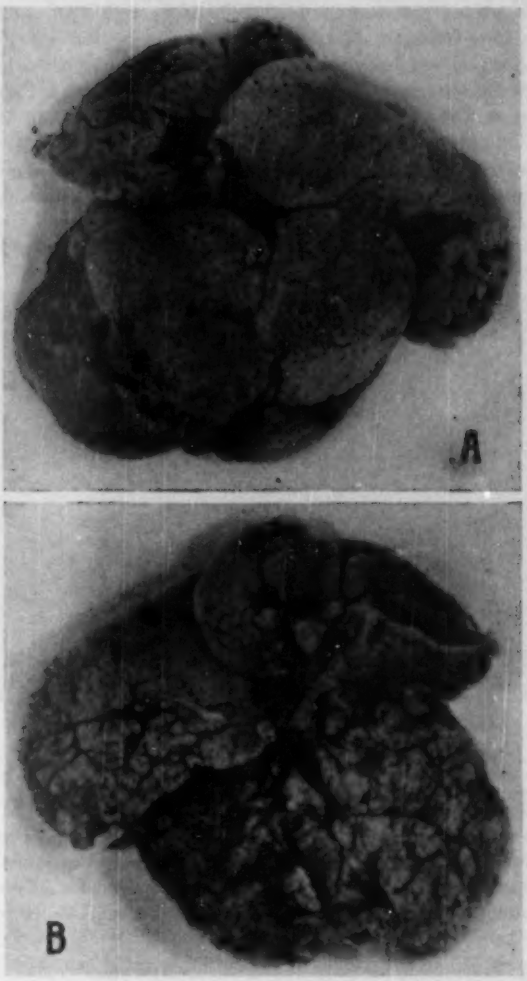

Fig. 12.—*A*, postnecrotic scarring. There are irregular, depressed, pigmented scars involving all lobes. There is no necrosis or hemorrhage. *B*, postnecrotic scarring. The under surface of the liver seen in *A* is shown.

cells, and considerable numbers of mitotic figures. Regeneration of tissue was more prominent in sections from the right and middle lobes than in those from the left lobe.

Sections stained for glycogen showed complete absence of glycogen granules in the necrotic cells whereas there were many prominent granules in those which escaped necrosis. The regenerating cells contained little glycogen.

Postnecrotic Scarring.—In the animals which survived the episodes of submassive necrosis there developed postnecrotic lesions which were extremely variable in gross and microscopic appearance. In a few

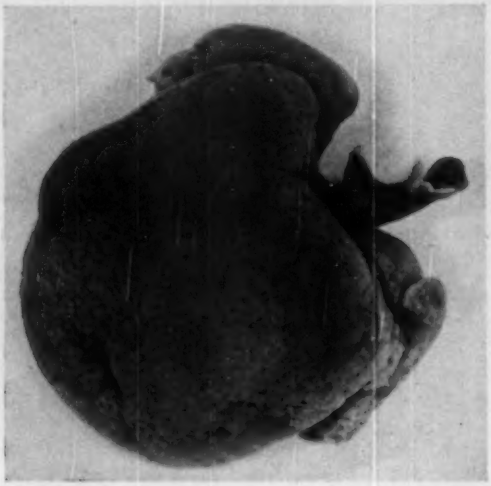

Fig. 13.—Postnecrotic scarring. There is extreme shrinkage of the left lobe which appears darker in color. The right half of the median lobe shows impressive hypertrophy.

instances there was no gross distortion of the liver. The majority of the livers, however, presented deep, irregular sulci which constituted bizarre deformities of the involved lobes (fig. 12). Nodules of yellowish regenerating liver tissue were sometimes prominent. The left lobe and the left half of the median lobe usually showed the greatest degree of involvement. This is particularly evident in figure 13, portraying a liver in which the entire left lobe was extremely shrunken, brown, firm and resistant to cutting. The right half of the median lobe, on the other hand, showed marked hypertrophy.

The microscopic examinations revealed the same variation in severity and distribution of lesions. In livers that on gross examination showed

little or no deformity, the scarred areas were more prominent about the central veins, with bands of fibrous tissue extending between the vascular areas. In some instances the fibrosis was limited completely to the central areas and consisted of loosely arranged fibroblastic scars in which there were numerous macrophages, some of which were pigmented. Grossly deformed livers showed dense fibrotic bands extending irregularly throughout the parenchyma, isolating groups of liver cells, with the production of a pseudolobular pattern (fig. 14). Some sections indicated a complete collapse of many lobules, which were replaced by a dense fibrotic tissue in which there were numerous macrophages and bile ducts. There were no necrotic or hemorrhagic areas. Evidence of regeneration

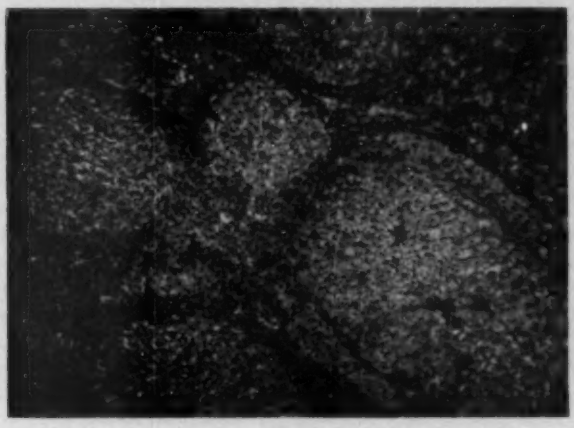

Fig. 14.—Section of a rat's liver showing postnecrotic scarring. Fibrotic bands extend irregularly throughout the section, isolating groups of liver cells, with a resultant pseudolobular pattern. Hematoxylin and eosin; × 90.

was not so prominent as in livers showing recurrent submassive involvement.

The glycogen content varied a great deal. It was essentially normal in many cells, but in others, particularly in the regenerating cells, it was decreased or absent.

COMMENT

Histologic changes in the liver similar to those observed by us prior to the development of necrosis have been described recently by Jaffé, Humphreys, Benditt and Wissler [20] in adult male rats fed diets low in

20. Jaffé, E. R.; Humphreys, E. M.; Benditt, E. P., and Wissler, R. W.: Arch. Path. 47:411, 1949.

protein and killed at intervals. Their rats consumed approximately 20 mg. of choline per day, which was considered adequate to supply the dietary requirements of labile methyl groups, and they concluded that the fatty change produced could not be attributed to a deficiency of choline.

Similar findings were also reported by Kosterlitz,[19b] who fed diets low in protein to adult rats and performed autopsies on them at intervals. He used the term "hypocytoplasmic" to describe the small, well defined liver cells which resulted from this treatment.

The decrease of cytoplasm and the disappearance of the basophilic granules of the liver cells are essential changes occurring prior to the development of necrosis. On the other hand, hepatic necrosis does not necessarily follow them, since they have been shown to occur in association with any deficiency of protein.[21] However, whether or not the progressive increase of stainable lipid observed in the prenecrotic livers is a prerequisite for the production of necrosis remains to be determined.

The grouping of livers that presented evidence of necrosis into the three divisions, massive necrosis, submassive and recurrent submassive necrosis, and postnecrotic scarring, is similar to that used by Himsworth and Glynn [7] with the exception that the term "submassive and recurrent submassive necrosis" have been used in place of the designation "partial necrosis." It is felt that these terms are more expressive of the type and degree of involvement and yet maintain a continuity of classification. György [22] used the term "acute diffuse necrosis" to describe extensive hepatic necrosis induced by dietary means, and this term may be considered synonymous with "massive necrosis."

The necrosis occurred suddenly after an interval of approximately 71 days, and if massive in character, the animal usually died within 24 hours. When, however, the necrosis was less extensive, that is submassive in character, the rats recovered after several days' illness, with the formation of irregular, pressed scars of the liver. Recurrent bouts of submassive necrosis resulted in death of the animals unless, of course, they were killed. Some of the animals which had experienced bouts of submassive necrosis were subsequently killed at a time when they were symptom free, and the livers of these animals displayed varying degrees of postnecrotic scarring.

The necrotic process was not zonal in type, since the liver lobules did not show a uniform zonal involvement. The cells about the central veins, however, appeared to be involved first, indicating that they were more susceptible to the damage of the liver. This observation was based

21. Kosterlitz,[19b] Jaffé and associates.[20]

22. György, P.: Am. J. Clin. Path. **14**:67, 1944.

on the findings in several of the animals killed when they were at the point of death. In addition to typical areas of well defined massive necrosis there were areas in which the only change observed under low magnification was an increased intensity of staining of the central and midzonal cells. However, under high magnification these cells showed nuclear pyknosis and karyorrhexis.

The initial attack of submassive necrosis usually involved the left lobe or the left half of the median lobe. This was clearly visible in the livers of 2 rats killed during the initial attack (fig. 8). Furthermore, in livers presenting evidence of recurrent necrosis the fibrosis and resulting deformity were more prominent in the left half of the liver. Himsworth and Glynn [7] explained that the initial attack of submassive necrosis was limited to the left half of the liver because of a striation of blood flow within the portal system, a phenomenon which has been repeatedly demonstrated, first by Serege (1901) in dogs, quoted and confirmed by Copher and Dick.[23] These workers showed that blood from the spleen, the stomach and the left colon passes primarily to the left half of the liver whereas blood from the small intestine and the right colon passes mainly to the right half of the liver. Glynn and Himsworth[24] demonstrated that in the rat the blood from the spleen was also distributed to the left lobe of the liver. They presented the theory that when diets deficient in the sulfur-containing amino acids are fed, these substances are absorbed from the small intestine and, although insufficient to give protection to the entire liver, they are sufficient to protect the right half, whereas blood from the spleen and the left colon, containing minimal amounts of these factors, offers no protection to the left half of the liver. This is an interesting and plausible explanation, but such an anatomic distribution of the necrotic process could also be explained on the basis of the distribution of an endotoxin produced by the spleen or the colon, as has already been suggested by Dent.[25]

SUMMARY

One hundred and twenty-one male rats which were fed a diet low in protein (7.2 per cent yeast protein) and deficient in alpha tocopherol were killed at intervals throughout an experimental period of 92 days. This diet was known to be capable of producing a high incidence of liver necrosis. The cytologic changes which occurred prior to and at the time of necrosis were studied by means of sections stained with hematoxylin and eosin, scarlet red, Best's carmine and Masson's trichrome stains.

During the prenecrotic period, the livers showed a prominent loss of cytoplasm and a decrease of basophilic granules. There was a pro-

23. Copher, G. H., and Dick, B. M.: Arch. Surg. **17**:408, 1929.
24. Glynn, L. E., and Himsworth, H. P.: J. Path. & Bact. **56**:297, 1944.
25. Dent, C. E.: Biochem. J. **41**:314, 1947.

gressive increase of stainable lipid in the liver cells about the central veins. Glycogen stains revealed little or no change except in sections where the central areas showed a marked increase of lipid. In such areas there was a decrease of stainable glycogen granules.

The livers which presented evidence of necrosis were divided on the basis of gross and microscopic observations into those showing massive necrosis, submassive and recurrent submassive necrosis, and postnecrotic scarring. These findings have been described in detail. Sections of the necrotic livers stained for fat yielded microscopic pictures so variable that no conclusions of a general nature could be formulated. As might be expected, the necrotic cells were devoid of glycogen.

HEPATIC NECROSIS INDUCED BY DIETARY MEANS

II. Biochemical Changes Occurring in the Liver During the Development of Necrosis

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ALTHOUGH considerable investigative work has been carried out on massive hepatic necrosis induced by dietary means, there have been relatively few reports on the chemical changes which occur during this process. Himsworth and Glynn [1] fed diets low in protein to a group of rats and compared the composition of the livers of animals in which necrosis developed with that of the livers of animals in which necrosis did not develop. They found that necrotic livers showed an increased concentration of water, an increase in the absolute amount of protein, no change in the level of nonprotein nitrogen and an absence of glycogen. On the basis of an estimation of total fatty acids plus unsaponifiable lipid, they concluded, "Hepatic necrosis occurred at all levels of liver fat and the affected animals showed the same proportions and amounts of liver fat as normal animals which had been taking the same diet for a similar length of time." Dent [2] determined the amounts of methionine and cystine in the livers of some of Himsworth's animals which displayed massive necrosis. He found that the methionine content was only slightly lower and the cystine content unchanged as compared with the contents of these amino acids in the livers of animals fed normal diets. The author concluded that the development of massive necrosis was not related to any change in the concentration of these amino acids in liver protein. However, up to the present time no systematic studies have been published with respect to the progressive biochemical changes occurring in the composition of the liver prior to and during the development of massive necrosis. It was felt that such a study might prove to be helpful in elucidating the pathogenesis of this lesion.

This investigation was financed by a grant from the National Research Council of Canada.

From the Departments of Pathology and Pathological Chemistry, Faculty of Medicine, University of Western Ontario.

1. Himsworth, H. P., and Glynn, L. E.: Biochem. J. **39**:267, 1945.

2. Dent, C. E.: Biochem. J. **41**:314, 1947.

EXPERIMENTAL PROCEDURE

A description of the diet and the treatment of the animals has been presented in the preceding paper. However, to summarize, 121 male rats were fed a diet low in protein, and groups of 5 animals were killed at intervals up to a period of 92 days. During the experimental period, a number of rats died of massive hepatic necrosis or were killed when near death. Each liver was analyzed for moisture, glycogen, total lipids, total fatty acids, total and free cholesterol, and phospholipids. Neutral fat and cholesteryl ester values were obtained by calculation.

Methods of Analysis.—Estimations of the glycogen contents of the livers of all killed animals were carried out on wedges of tissue from the three main lobes taken immediately after killing, according to the method described by Good, Kramer and Somogyi.[3] Nelson's[4] procedure for the estimation of dextrose was utilized. After thin slices of tissue were taken from representative areas of each lobe of the liver for the preparation of histologic sections, the remaining portions, which comprised 50 to 60 per cent of the total liver weight, were homogenized with 10 volumes of acetone in a small Waring blendor[*] for a period of 30 seconds. The mixture was centrifuged and the residue reextracted four times with five volumes of hot 95 per cent alcohol. The combined extracts were made up to volume (usually 150 or 200 cc.) with acetone-alcohol (1:1). This method has previously been shown by Lucas[5] to effect an extraction of more than 99.5 per cent of the lipids present.

Although slight modifications were made in the methods for determining the lipids, the procedures followed were essentially those described by Sperry and Brand[6] for total cholesterol, by Sperry[7] for the Schoenheimer-Sperry method for free cholesterol and by Sinclair and Dolan[8] for phospholipid. In the last method, however, the phospholipid phosphorus was determined according to the procedure of Beveridge and Johnson.[9] Total lipids were determined by evaporating an aliquot of the lipid extract to dryness, reextracting the residue with petroleum benzin, evaporating off the solvent and weighing the dry material. This fraction was saponified in the usual manner, acidified and the total fatty acids extracted with petroleum benzin. After evaporation of the solvent, the amount of the total fatty acid fraction was obtained by weighing the residue.

All these methods yielded closely agreeing replicate values on samples of lipid extract. In Sperry's procedure for free cholesterol, slight modifications were introduced simplifying and facilitating the performance of the determination (Beveridge and Johnson[10]).

The lipid-free liver residue was dried to approximately constant weight by placing it in an oven at 90-95 C. for 12 hours at a time. After the second drying

3. Good, C. A.; Kramer, H., and Somogyi, M.: J. Biol. Chem. **100**:485, 1933.

4. Nelson, N.: J. Biol. Chem. **153**:375, 1944.

5. Lucas, C. C.: Personal communication to the authors, 1945.

6. Sperry, W. M., and Brand, F. C.: J. Biol. Chem. **150**:315, 1943.

7. Sperry, W. M.: The Schoenheimer-Sperry Method for the Determination of Cholesterol, Department of Biochemistry, New York State Psychiatric Institute and Hospital, New York, 1943.

8. Sinclair, R. G., and Dolan, M.: J. Biol. Chem. **142**:659, 1942.

9. Beveridge, J. M. R., and Johnson, S. E.: Canad. J. Research, Sect. E **27**:159, 1949.

10. Beveridge, J. M. R., and Johnson, S. E.: Brit. J. Exper. Path., to be published.

period the weight of the residue was usually within 1 per cent of that noted after the first period of drying. Each lipid value was expressed as the ratio of the lipid weight to the dry, fat-free liver weight.

RESULTS

The classification of the necrotic livers according to structural findings into massive necrosis, submassive and recurrent submassive necrosis, and postnecrotic scarring, has been explained fully in the previous paper (Abell, Beveridge and Fisher [11]). In the accompanying charts the term "submassive necrosis" includes livers of animals showing both submassive and recurrent submassive involvement.

Of 8 livers exhibiting postnecrotic scarring, only 5 were analyzed. The results obtained therefrom were of such a variable nature that for purposes of simplification they have not been included in the charts. For the sake of completeness, however, they are given in the table following the presentation of the more significant and consistent data.

Wet Liver Weight (chart 1*a*).—A sharp initial decrease in wet liver weight (expressed as a percentage of final body weight [12]) occurred during the first 15 days. Following this, no change took place during the prenecrotic period. During the time at which necrosis developed in some of the animals, the wet weights of the livers of rats killed at random increased but did not attain the values found for the normal control rats. The weights of livers showing massive necrosis were increased sharply above the levels in the normal control rats, whereas the weights of those showing submassive involvement also were increased but not so consistently nor to the same degree.

Dry Liver Weight (chart 1*b*).—The dry weight of the liver (expressed as a percentage of final body weight) paralleled almost exactly the wet liver weight except in the cases of hepatic necrosis.

In the presence of submassive involvement the dry liver weights were actually lower than those of the livers showing no necrosis from rats killed during the same interval. On the other hand, the livers which exhibited massive necrosis contained on the average definitely greater amounts of nonaqueous material than the other livers with the exception of those of the control animals. It may be noted that this increase in dry liver weight was not as great proportionately as the increase in wet liver weight.

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11. Abell, M. R.; Beveridge, J. M. R., and Fisher, J. H.: Arch. Path., this issue, p. 1.

12. Since the wet and dry liver weights have been expressed as percentages of final body weight, any marked change in the latter immediately prior to death or killing would lead to artificial changes in these values. However, there were no such significant fluctuations in body weight, and hence any changes noted may be referred specifically to the liver and are not a reflection of variations in body weight.

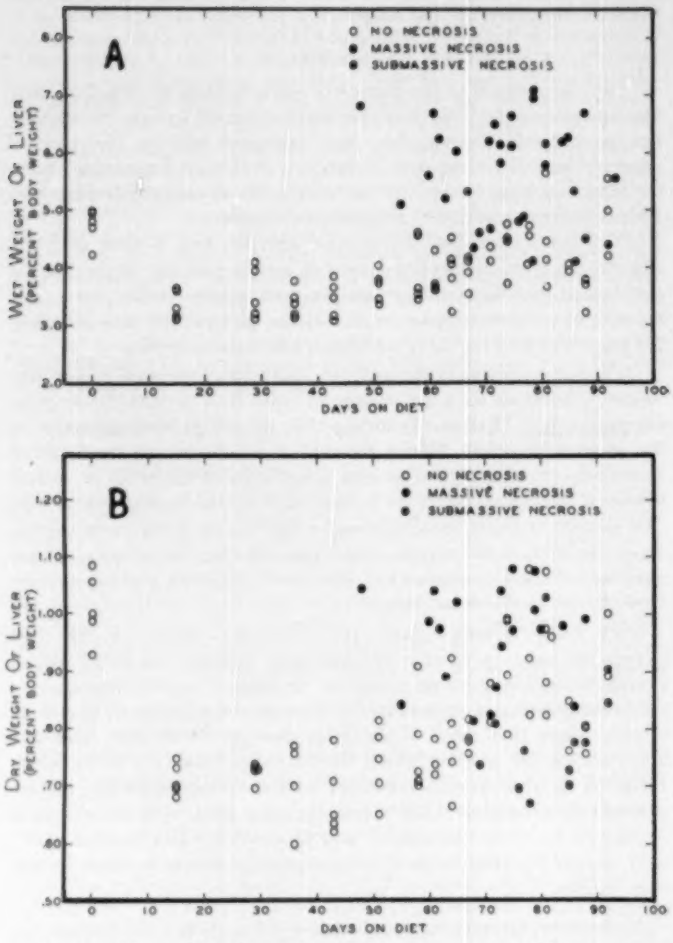

Chart 1.—Liver weights in the absence of hepatic necrosis and in the presence of massive or of submassive necrosis: *A*, wet liver weights; *B*, dry liver weights.

Water Content of Liver (chart 2).—The water content of the liver (expressed as a percentage of wet liver weight) remained essentially constant in all non-necrotic livers. Those presenting evidence of massive

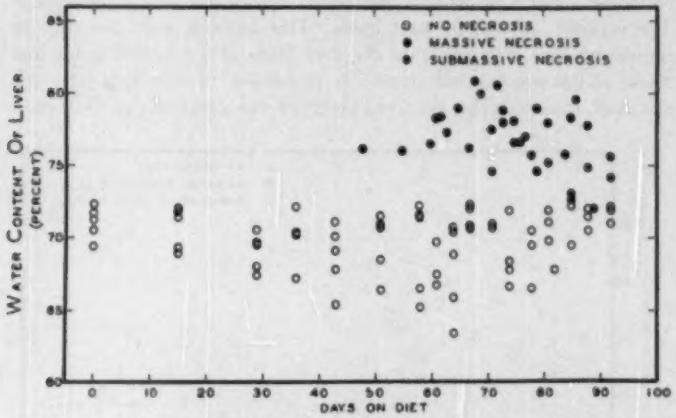

Chart 2.—Water component of the liver (evaluated as percentage of wet liver weight) in rats showing no hepatic necrosis, those showing massive necrosis and those showing submassive necrosis.

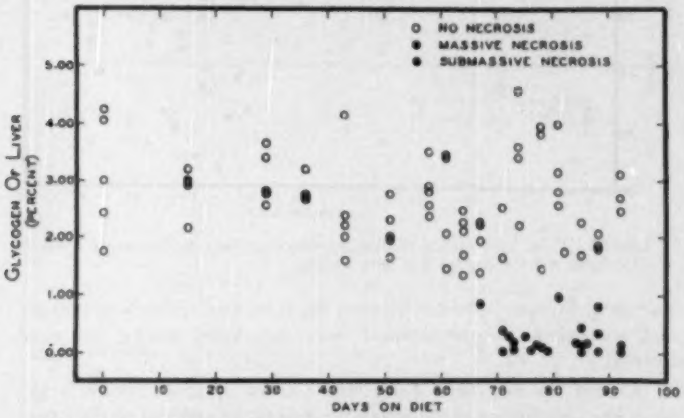

Chart 3.—Glycogen component of the liver (evaluated as percentage of wet liver weight) in rats showing no hepatic necrosis, those showing massive necrosis and those showing submassive necrosis.

or submassive necrosis had a definite increase in water content, which was greater in the former type of lesion.

Glycogen Content of Liver (chart 3).—The glycogen content of the liver (expressed as a percentage of wet liver weight) did not change

materially in the non-necrotic livers. As might have been predicted, however, the glycogen values fell to extremely low levels in the necrotic livers, especially in those showing massive involvement.

Total Lipid Content of Liver (chart 4).—There was a progressive but variable increase in total lipids. This increase was shown to be significant by a comparison of the liver lipids of the control group and those of the non-necrotic livers. A probability of less than 0.05 was obtained, indicating that the trend observed was significant. It is obvious

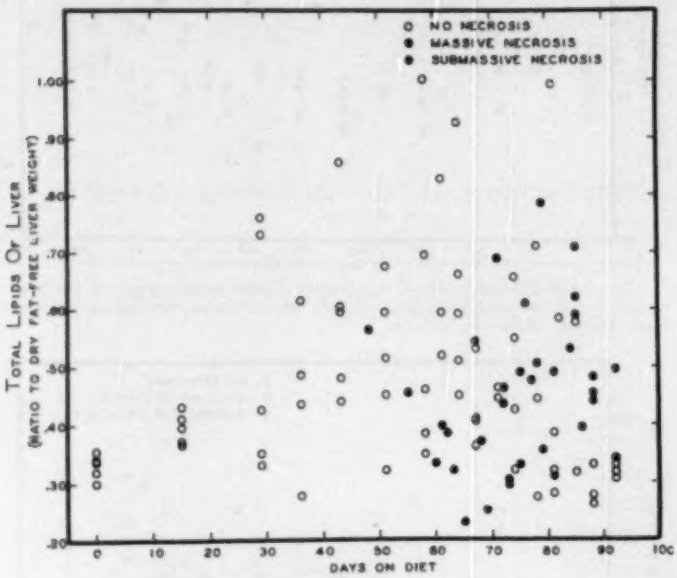

Chart 4.—Total lipid content of liver, expressed as the ratio between the weight of total lipids and the dry fat-free liver weight.

that there was no difference between the total lipid contents of necrotic and non-necrotic livers obtained from rats killed during the same interval.

Neutral Fat and Total Fatty Acid Content of Liver (charts 5 and 6).—The distribution of the values for neutral fat and that of the values for total fatty acids paralleled that already shown for total lipid, and essentially the same conclusions may be drawn.

Phospholipid Content of Liver (chart 7).—Phospholipid levels remained practically unchanged except in the necrotic livers. In those

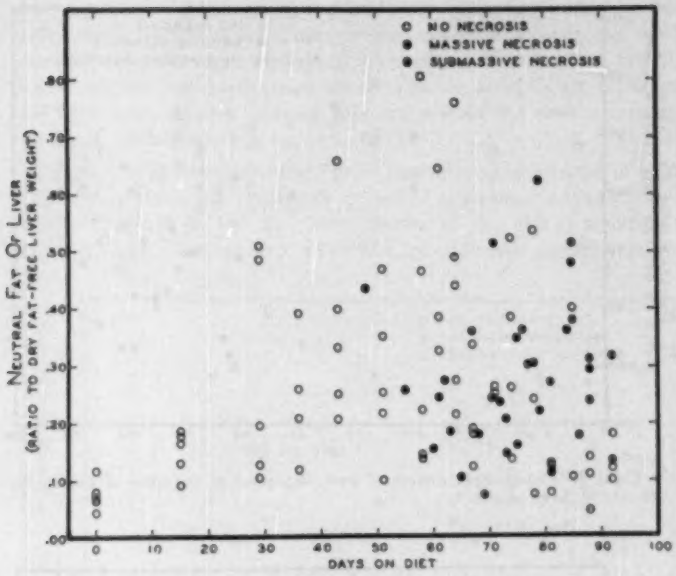

Chart 5.—Neutral fat content of liver, expressed as the ratio of this to the dry fat-free liver weight.

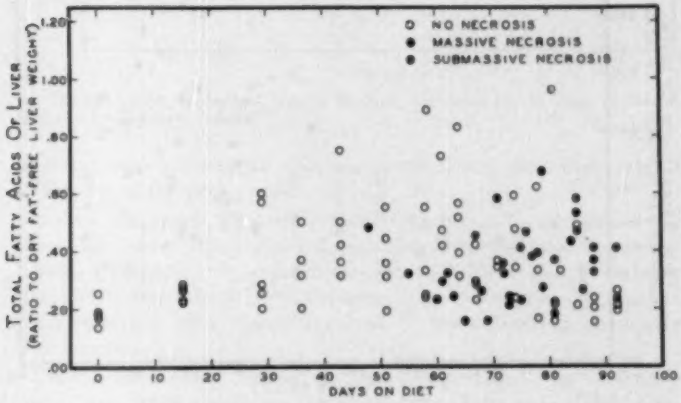

Chart 6.—Total fatty acid content of liver, expressed as the ratio of the weight of the fatty acids to the dry fat-free liver weight.

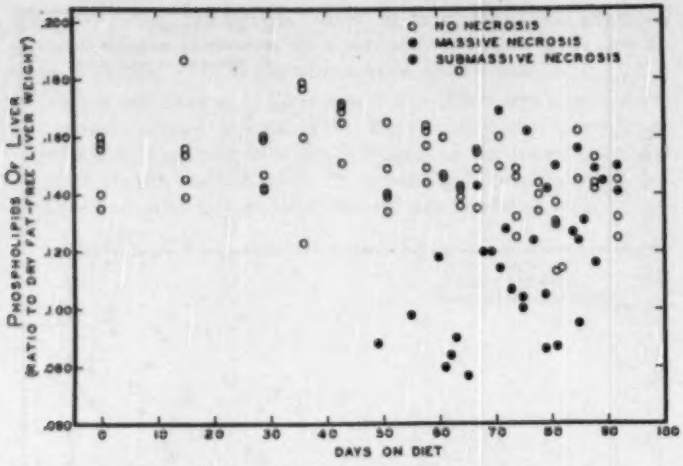

Chart 7.—Phospholipid content of liver, expressed as the ratio of this to the dry fat-free liver weight.

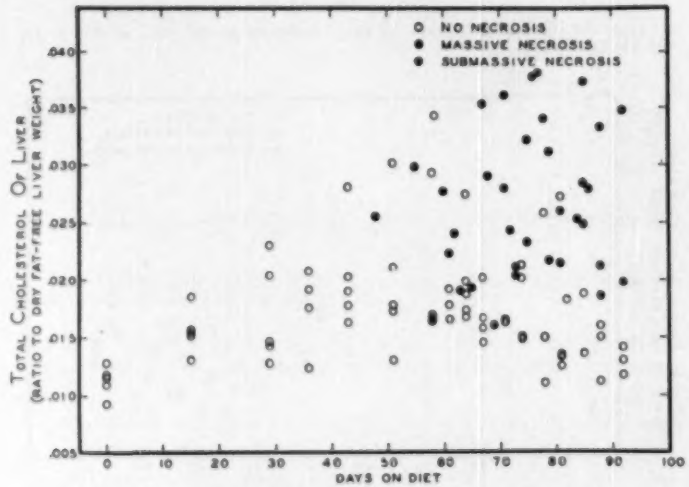

Chart 8.—Total cholesterol content of liver, expressed as the ratio between this and the dry fat-free liver weight.

showing massive involvement they were obviously decreased. On the other hand, no significant difference was observed between the non-necrotic livers and those displaying submassive necrosis. These results would suggest that the decrease observed in the phospholipid levels of the livers with massive necrosis does not precede the development of the lesion but actually is a sequel to it.

Total Cholesterol Content of Liver (chart 8).—The increase in total cholesterol during the prenecrotic period is apparent from an examination of chart 8 and has been shown to be highly significant ($P = <0.01$). Furthermore, there was an additional elevation in all

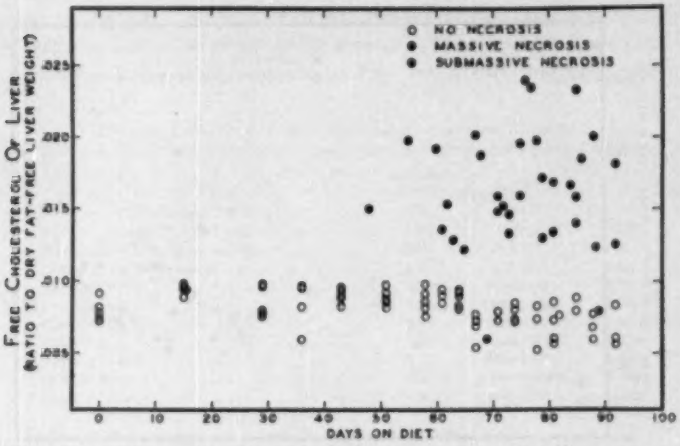

Chart 9.—Free cholesterol content of liver, expressed as the ratio of this to the dry fat-free liver weight.

necrotic livers as compared with non-necrotic livers taken from animals killed during the same period.

Free Cholesterol Content of Liver (chart 9).—In all non-necrotic livers the level of free cholesterol remained unchanged from the control values throughout the experimental period. There was a dramatic increase, however, in all livers displaying massive necrosis, and with two exceptions the same change occurred in livers showing submassive involvement.

Cholesteryl Ester Content of Liver (chart 10).—That cholesteryl ester levels are increased in the non-necrotic livers over those in the control livers is obvious. Since it has been shown previously that the levels of free cholesterol remained unchanged in these livers, it may be

concluded that the increase of total cholesterol in the non-necrotic livers was due entirely to the increase in cholesteryl ester. It is also obvious that there was no difference in cholesteryl ester levels between the non-necrotic and the necrotic livers. Hence the difference in total cholesterol values between these two groups of livers was due solely to an increase of free cholesterol in the necrotic livers.

The analytic results from the postnecrotic livers were, as previously stated, somewhat variable in nature. This may be explained, at least in part, by the differences in the degree of fibrosis and the extent of the associated loss of parenchymal tissue. The differences noted between the analytic data from the postnecrotic and those from the necrotic livers

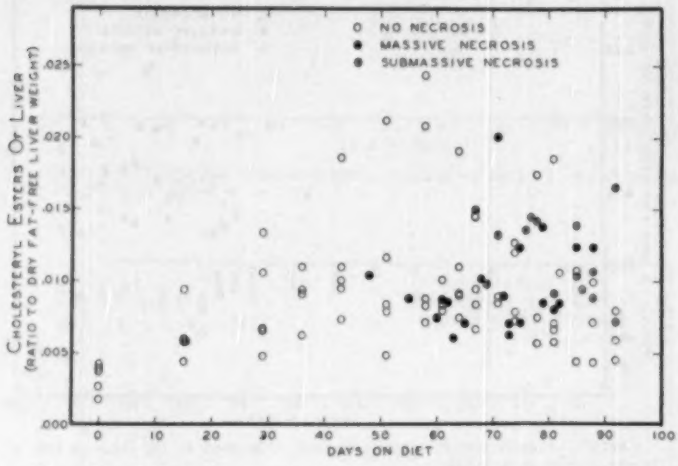

Chart 10.—Cholesteryl ester content of liver, expressed as the ratio of this to the dry fat-free liver weight.

were probably due to a combination of two factors—the increased amount of fibrous tissue associated with the previous destruction of parenchymal cells and the absence of active necrosis at the time of autopsy. When the data in the table are compared with those in charts 1 to 10 there are revealed certain differences and similarities. The wet liver weights and water contents of the postnecrotic livers were greater than those of the non-necrotic livers but lower than those of the groups that showed necrosis. The dry liver weights were intermediate between those of massively necrotic and those of non-necrotic livers. Glycogen values, although depressed as compared with those of non-necrotic organs, did not reach the extremely low values seen in the necrotic

group. The phospholipid levels were greater than the decreased values observed in massive necrosis but nevertheless were definitely lower than the figures obtained for the other livers. The values for total lipids, total fatty acids and neutral fat, although elevated, were lower than those of livers showing massive, submassive or no necrosis. The cholesterol components fell within the same range as those from the non-necrotic livers.

COMMENT

The initial proportional decrease in wet and dry liver weight indicated a loss of material, presumably protein, along with a proportional amount of water. Associated with this change was a perceptible decrease of cell size and a loss of basophilic granules. This occurrence has been demonstrated by other workers in rats fed a diet low in protein

Analytic Data from Livers Showing Postnecrotic Scarring

Analysis	Average Values	Range	S. D.
Wet liver weight (% body weight)	4.31	3.93-4.57	0.244
Dry liver weight (% body weight)	0.829	0.738-0.911	0.079
Water content (% liver weight)	73.50	72.5-76.2	1.20
Glycogen (% liver weight)	1.86	0.00-3.52	1.294
Total lipid *	0.390	0.290-0.646	0.150
Neutral fat *	0.210	0.0965-0.462	0.166
Total fatty acid *	0.350	0.179-0.569	0.0496
Phospholipid *	0.127	0.098-0.156	0.0205
Total cholesterol *	0.178	0.0128-0.0242	0.00481
Free cholesterol *	0.00991	0.00658-0.01220	0.00365
Cholesteryl ester *	0.00869	0.00352-0.01380	0.0038

* Lipid values are expressed as ratios to the dry fat-free liver weight.

(Kosterlitz [13]; Jaffé, Humphreys, Benditt and Wissler [14]). The latter workers showed by chemical analysis that the protein component of the liver was decreased by such diets. The increase in total lipids was readily correlated with an increase in stainable lipid, which was seen to appear primarily around the central veins, thereafter gradually spreading toward the periphery of the hepatic lobule. This change in liver composition is similar to that reported by the authors just mentioned. The phospholipid content remained essentially unchanged. Kosterlitz [13] reported a decrease of this liver conponent, but his data were calculated on the basis of wet liver weight whereas those of this paper were cal-

13. Kosterlitz, H. W.: J. Physiol. **106**:194, 1947.

14. Jaffé, E. R.; Humphreys, E. M.; Benditt, E. P., and Wissler, R. W.: Arch. Path. **47**:411, 1949.

culated on the basis of fat-free dry liver weight. Since water and fat content of liver may change so quickly and extensively, it is felt that the latter procedure, as fully explained previously by Beveridge and Lucas,[15] offers a much more accurate picture of changes in liver composition. The decrease of liver phospholipid found by Kosterlitz is due, as shown by the values presented here, to a fatty infiltration of the liver, which causes an apparent decrease in other liver components. When our data are expressed on the same basis as those of Kosterlitz, a similar result is obtained. Since phospholipid and free cholesterol remained constant in the prenecrotic livers, it may be concluded that the increase in total lipids observed during the prenecrotic period was due to an elevation of cholesteryl ester and neutral fat.

The sharp increase in weight of livers showing massive necrosis was due largely to an influx of fluid but also to an influx of nonaqueous material. Himsworth and Glynn [1] demonstrated that part of the increase may be attributed to protein. They also found that glycogen fell to extremely low levels whereas total lipids remained essentially unchanged as compared with those of non-necrotic livers obtained from animals restricted to the test diet for a similar length of time. Confirmation of these results is contained in the present report.

Two additional dramatic changes have been demonstrated in the composition of necrotic livers in the present work. Phospholipid fell precipitously whereas free cholesterol increased markedly. The fall of phospholipid was found only in those showing massive damage, and the rise of cholesterol was found in both massive and submassive necrosis. Both glycogen and phospholipid are well known to be rather labile substances and the significant decreases noted might be explained on the basis of death of tissue and concomitant destruction of these compounds. Emulsions of liver tissue have been shown by Sperry and Brand [16] to be active in the esterification of cholesterol. Thus the increase of this component found in the organ at the time of necrosis may be due to a failure of this aspect of liver function and a consequent accumulation of the free sterol.

Work is now in progress to determine whether or not the changes in liver composition observed during the prenecrotic phase and at the time of necrosis are inevitable features of the development of the hepatic lesion.

SUMMARY

One hundred and twenty-one male rats which were fed a diet low in protein (7.2 per cent yeast protein) and deficient in alpha tocopherol were killed at intervals throughout an experimental period of 92 days.

15. Beveridge, J. M. R., and Lucas, C. C.: J. Biol. Chem. **157**:311, 1945.
16. Sperry, W. M., and Brand, F. C.: J. Biol. Chem. **137**:377, 1941.

During the prenecrotic period there was an initial and proportional fall in both dry and wet liver weight. Total lipids, neutral fat, total fatty acids and cholesteryl esters showed a progressive increase during this period but no additional significant change at the time of necrosis. Glycogen and phospholipid levels remained unchanged in all non-necrotic livers.

The massively necrotic livers differed from the non-necrotic that were obtained from animals killed during the same interval by a significant increase in both wet and dry liver weight, striking increases in water and free cholesterol and equally striking decreases in phospholipid and glycogen levels. With the exception of the phospholipid component, which was essentially unchanged, the foregoing statement is also applicable to livers showing submassive necrosis.

Dr. Shirley E. Johnson performed some of the analytic determinations.

CEREBELLAR DISORDERS IN LAMBS

A Study in Animal Neuropathology with Some Comments on Ovine Neuroanatomy

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I. GENERAL INTRODUCTION

STUDIES of animal nervous diseases are primarily of veterinary importance, but they also can help to clarify human neuropathologic conditions. More attention might be paid with advantage to the modern technics elaborated by medical neurologists, for if more meticulous methods of study of animal conditions (clinical and pathologic) had been used in the past, some nervous diseases might have been identified years before they actually were. Many fatal and nonfatal nervous diseases of animals may still await clarification, since different morbid entities could be confused clinically, while many established disorders of known and unknown cause might still be investigated profitably with more detailed pathologic study. The forms of disease to be described are illustrative in these directions; "daft lambs," for instance, must have occurred for years before pathologic identification.

The studies reported herein partly emanated from work on sway-back—a paralysis of lambs in which cerebral demyelination is found. (See review by Innes.[1]) "Paralysis" of young lambs has, however, often been a source of clinical confusion. For example, we have found sway-back mistaken for cerebellar degeneration or abiotrophy, cerebellar atrophy for sway-back and bilateral myopathy, purulent vertebral osteomyelitis and meningomyelitis for sway-back, and myelitis following tick bite infection for sway-back. This need not be regarded as surprising (or derogatory to the skill of the veterinarian), because farmers may never consult veterinarians in regard to diseased sheep, and, furthermore, limitations of neurologic diagnosis of animal diseases are

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1. Innes, J. R. M.: Demyelinating Diseases of Animals, in symposium on Multiple Sclerosis, New York, 1948, A. Research Nerv. & Ment. Dis., Proc. **28**: 75-98, 1950; Vet. Med. **44**:340, 421 and 467, 1949.

placed on veterinarians, who might be inclined to rely more on history of the flock than on careful observation of individual animals. This is influenced by economics, which enters into all spheres of veterinary work, a fact which accounts somewhat for the apparent paucity of pathologic details of many animal diseases; it may simply not be worth the time, the trouble or the expense to investigate sick animals if only a few are affected

Cerebellar atrophies occur in a number of species, and we have observed them not only in lambs but also in man, calves, kittens and puppies, but this study is limited to lambs. Some study of the normal cerebellum of lambs is necessary as a basis for this work, and brief comments on the normal neuroanatomy are advantageous.

NORMAL ANATOMY AND HISTOLOGY OF THE LAMB'S CEREBELLUM

Ovine neuroanatomy has not been studied so minutely as that of man, the cat, the dog or the pig as can be seen by reference to Ariëns Kappers, Huber and Crosby [2] (1936); the only general account (Wilkie,[3] 1937) is superficial and meant for young students of comparative anatomy and zoology. Other neuroanatomic references include the papers by Dexler and Margulis [4] (1900) and King [5] (1911) on the motor paths,[5a] by Simpson and King [6] (1911) and Bagley [7] (1922) on the motor area, by Anthony and Grzybowski [8] (1936-1937) on development, and by Pattison and Holman [9] (1943) on the location of the cranial nerve nuclei.

The cerebellum of the newborn lamb (or adult sheep) is relatively small. From the dorsal aspect the median lobe is prominent, but the division between this and the two lateral hemispheres is not so obvious as in man. Figure 1 *A* and *B*, shows the primary fissure and the prominence of the anterior and middle parts of the median lobe, which curves on itself to disappear between the ansiform lobes; lateral to this and the pontile pillar is the flocculus, which is large in the sheep,

2. Ariëns Kappers, C. U.; Huber, G. C., and Crosby, E. C.: The Comparative Anatomy of the Nervous System of Vertebrates Including Man, New York, The Macmillan Company, 1936.

3. Wilkie, J.: The Dissection and Study of the Sheep's Brain as an Introduction to the Study of the Human Brain, London, Oxford University Press, 1937.

4. Dexler, H., and Margulis, A.: Morphologisches Jahrbuch, Leipzig **35**: 143, 1900.

5. King, J. L.: Quart. J. Exper. Physiol. **4**:133, 1911; J. Comp. Neurol. **21**:311, 1911.

5a. There is a need for meticulous work on ovine neuroanatomy. These workers showed that the motor area of the sheep is restricted to the superior frontal gyrus and that the sheep possesses a relatively small pyramidal tract, most of the fibers of which decussate in the lower part of the medulla. As a result of hemisection lesions, two other well developed descending tracts can be demonstrated, one probably corresponding to the rubrospinal tract of the cat.

6. Simpson, S., and King, J. L.: Quart. J. Exper. Physiol. **4**:53, 1911.

7. Bagley, C. Jr.: Arch. Neurol. & Psychiat. **22**:417, 1922.

8. Anthony, R., and de Grzybowski, J.: J. Anat. **71**:41, 1936-1937.

9. Pattison, I. H., and Holman, H. H.: J. Comp. Path. & Therap. **53**:130, 1943.

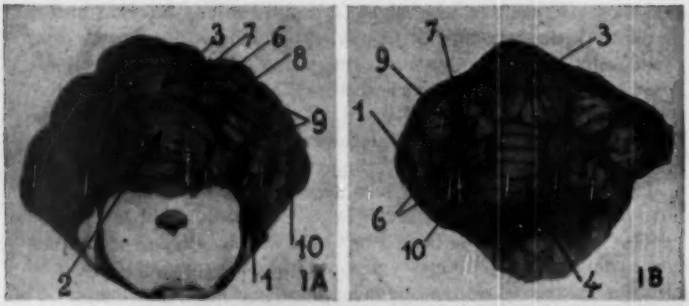

Fig. 1.—Cerebellum of normal newborn lamb. *A*, cranial view, and *B*, caudal view with small portion of cortex removed on the right. In this and the following figure *1* indicates the paraflocculus; *2*, anterior part of the median lobe; *3*, middle part of the median lobe; *4*, posterior part of the median lobe; *5*, lingula; *6*, paramedian lobe; *7*, paramedian fissure; *8*, primary fissure; *9*, ansiform lobe (*9a*, crus I, and *9b*, crus II); *10*, flocculus.

Fig. 2.—Cerebellum of normal newborn lamb: vertical transverse sections. Weigert-Pal; × 7.

Fig. 3.—Cerebellum of normal newborn lamb: dentate nucleus and roof nuclei. Formaldehyde solution, pyroxylin, cresyl violet; × 17.

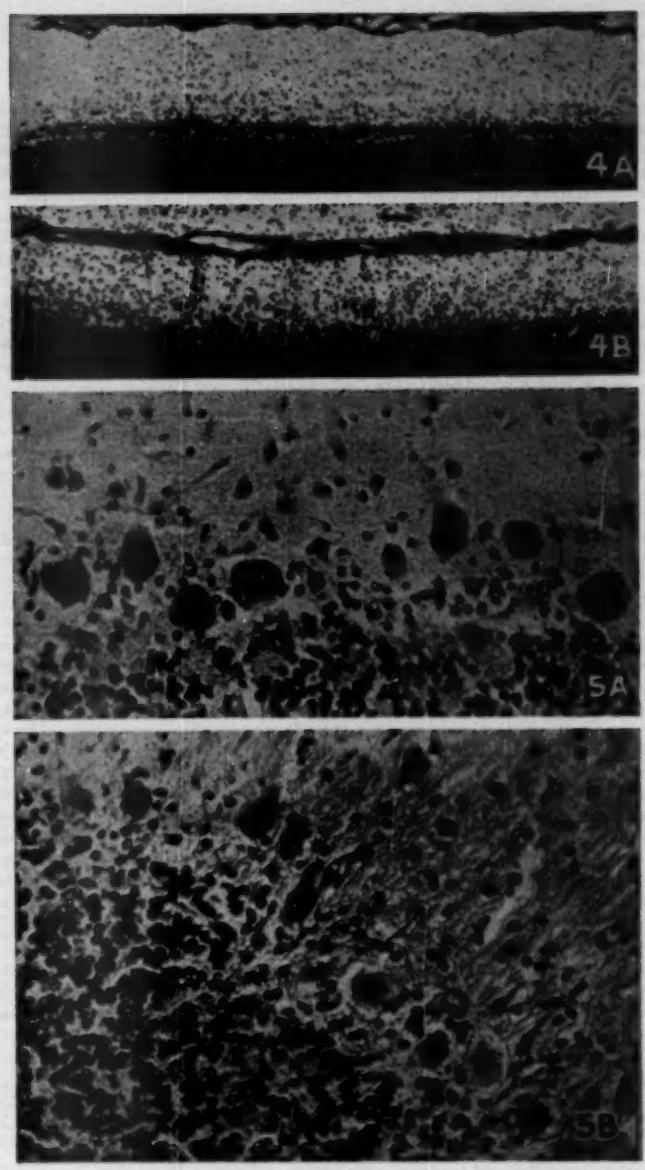

Fig. 4.—*A*, cerebellum of normal lamb, showing relatively regular arrangement of Purkinje cells. *B*, cerebellum of "daft lamb" (case 12), showing slight reduction in volume of the molecular layer, decided loss of Purkinje cells and glial proliferation. Paraffin, Nissl; × 55.

Fig. 5.—*A*, cerebellum of normal lamb. *B*, atrophied cerebellum of "daft lamb" (case 6), showing initial changes in tinctorial affinity of Purkinje cells—some dark stained and shrunken and others pale and granular or vacuolated—and discrete glial proliferation from the Bergmann layer. Paraffin, Nissl; × 120.

and above this is the paraflocculus. Viewed from behind are the folds formed by the middle and posterior parts of the median lobe. In figure 1*B* a small part of the cortex has been removed to reveal the relationship of the flocculus and the paraflocculus to the paramedian lobe. Figure 2 is a verticotransverse section which facilitates further identification and is helpful in following later descriptions.

In the newborn lamb the cerebellum has a molecular layer poor in glia except in the subpial zone; in the latter there is a layer of small cells with deeply stained round nuclei and without visible cytoplasm—the remains of the extrasubpial granular layer (Obersteiner's layer—superficial cortex) which disappears within a few weeks of birth. The Purkinje cells (fig. 5 *A*) are round or piriform and are arranged relatively compactly (fig. 4 *A*); like those of other mammals they have a vesicular nucleus, a cytoplasm filled with Nissl granules and a pale apical dendron extending into the molecular layer. Their density might depend on section thickness; in paraffin sections it is normal to find Purkinje cells separated by a space having the diameter of about 2 or 3 cells, while in thicker celloidin (pyroxylin) or frozen sections they may appear much closer packed. There is much variation in different regions, and it is not unusual to find gaps with no Purkinje cells, equal to the diameter of 5 to 6 cells or even much bigger. In the cerebellum of many normal adult sheep folia are often seen with areas in which there is a relative paucity of Purkinje cells, which emphasizes that "the extent of normality" requires rigid definition when pathologic states are being interpreted. Neuroglial cells (Bergmann), of which only the round or the spindle shaped nuclei are seen, may be so dense in the Purkinje cell layer in celloidin sections as to appear pathologic. Golgi cells penetrate deeply into the granular layer and are oval or round, with clear nuclei, which are often superimposed on one another in sections; the cytoplasm contains Nissl granules. The neuroglia cells at the Purkinje cell level are of two types; the nucleus of one type is small and deeply stained and that of the other (the more numerous type) is large and clear. Myelin stains show good development of myelinated fibers in lobes, lobules and folia. Using Cajal's gold chloride–mercuric chloride method after formaldehyde–ammonium bromide or formaldehyde fixation, we obtained excellent impregnations of astrocytes with truncated branched processes in the granular layer, but in the same preparation normal glia of the molecular layer remained unstained. The white matter has a relative paucity of glia, and here it was easier to impregnate astrocytes with Hortega's than with Cajal's method. Although no study was made of the distribution of glia in the ovine nervous system, excellent demonstrations of astrocytes, microglia and oligodendroglia have been obtained in many parts by silver methods after formaldehyde fixation. In scarlet red preparations there is little lipoid material in neurons or glia. The dentate nucleus is a triangular-shaped mass with its apex below and with deeply stained angular, oval or polygonal neurons, between which are numerous neuroglia cells with the clear nuclei, and the capillary network is relatively dense (fig. 3). (The roof nuclei show the same structure.) In Cajal preparations astrocytes were as numerous there as in the granular layer.

The brain of the newborn lamb weighs around 75 Gm., and that of the adult sheep not much more than 90 Gm., so that relatively little postnatal growth occurs. Information concerning myelination apart from the work of Romanes[10] (1947) is scant, and it is pertinent to note that the lamb rises and walks within a few seconds after birth. Romanes showed that cerebral myelination was almost fully developed at birth and cerebellar myelination earlier; he found that myelination

10. Romanes, G. J.: J. Anat. **81**:64, 1947.

began in the uncinate fasciculus, the dorsospinal cerebellar tract and the medullated fibers of the cortex vermis around sixty-six days of gestation, in the ventrospinal cerebellar tract and the brachium conjunctivum at seventy-eight days and in the cortex of the lateral lobes and the brachium pontis at ninety-six days. The spatial arrangement of the Purkinje cells must be well established half through gestation (sixty to seventy days) and probably much earlier. Examination of a limited number of sections of cerebellum of fetal lambs of known ages (presented by Dr. G. Romanes) indicated that cellular migration was well forward by one hundred days, with appearances of full differentiation; the temporal order of development may be of significance in any explanation of the formal genesis of the disorders to be described.

II. "DAFT LAMBS"—INHERITED CORTICAL CEREBELLAR ATROPHY OF NEWBORN LAMBS IN BRITAIN

White and Rowlands [11] (1945) recorded a "hereditary defect of lambs" occurring in North Wales and known locally as "daft lambs"; they dealt with the history and the signs which were said to resemble those of sway-back. The pathologic changes were not established by them, but the brains were reported as macroscopically normal. Subsequently, specimens were received from Rowlands for pathologic study; before and after this time other lambs were obtained from different parts of Britain with a similar history and signs. Innes, Rowlands and Parry [12] (1949) dealt in a preliminary way with the disorder, sufficient to permit its identification by others, and concluded that it was a form of cerebellar atrophy which might be commoner than suspected because of the possibility of its being clinically confused with sway-back and other "paralyses."

Fifteen reputed "daft lambs" were collected in Britain, 9 of which were seen alive by one of us (J. R. M. I.); some of the lambs were kept alive by artificial feeding and died or were killed at times extending from a few days up to a month after birth. Only carcasses of 2 were obtained, and in 4 lambs the examination was limited to the nervous system. Of these 15 cases, 13 were finally placed in this category of atrophy described in part II; these were obtained from Scotland (1), North Wales (8), Northumberland (2) and Yorkshire (2); the remaining 2, of a different nature, are treated in an appendix to this paper.

CLINICAL OBSERVATIONS

The mothers of affected lambs were healthy, with normal gestation and parturition. The affected progeny showed variation in severity of the signs. They were mostly single lambs, but occasionally there were twin lambs, only one of which was affected. Some walked with difficulty and achieved this by straddling their legs on a wide base (fig. 6*C*).

11. White, R. G., and Rowlands, W. T.: Vet. Rec. **57**:491, 1945.

12. Innes, J. R. M.; Rowlands, W. T., and Parry, H. B.: Vet. Rec. **61**:225, 1949.

There was a tendency toward wandering without purpose, toward some circling movements or staggering backward before collapsing, and rising was difficult. The lambs bleated feebly, sucked vigorously from a bottle, followed it if held near the nose, and wagged their tails when sucking. There may have been defective vision which, combined with faulty balance, could have caused them to stumble off, rather than to walk off, a low table. In some there were opisthotonos (fig. 6*B*) and coarse tremors. Eye movements and corneal and pupillary reflexes were normal. Excessive noise did not disturb them. In 1 lamb (no. 9), for example, kept alive for a few weeks, the signs did not progress until near death, when there was general weakness.

Some lambs lay helpless on their side but with head posture normal for lying (fig. 6*A*); from the photographs of such "reclining paralysed"

Fig. 6.—*A*, "daft lamb" 6 days old (case 6) unable to rise; the reclining posture is normal, and the animal is able to hold its head up; there is some rigidity of the forelimbs. *B* and *C* (enlargements from 16 mm. motion picture film), another "daft lamb" (case 9), showing (*B*) opisthotonos and (*C*) broad-straddled base when standing.

animals it would be difficult to detect any abnormality. The head-righting and neck-righting reflexes were normal. When one held the lamb by the body, coarse tremors were sometimes seen. The limbs were moved voluntarily but could not be placed beneath the body for standing, and if the lamb was placed on its feet, there was insufficient tone to maintain position. Some spasticity of the limbs was noted. There was increased response to pressure on the sole of the foot but compression (pain) of the digits elicited a normal withdrawal reflex. The patellar reflex seemed exaggerated.

Under natural conditions severely affected lambs would soon die from exposure, lack of food or secondary infection if they were not killed

by the farmer when noticed to be born paralyzed (a customary practice of farmers is not to rear defective animals). The existence of such conditions might thereafter be ignored by the farmer unless the losses became severe, and this indifference might be one reason why the disease has not been identified previously and why the studies made on many other heredopathologic conditions of farm animals have been inadequate.

"Daft lambs," therefore, showed signs of hypotonia, asthenia, abnormality of muscle tone, difficulty in equilibration and sometimes tremors; nystagmus was absent and pupillary inequality was seen only occasionally.

GENETIC HISTORY

A breeding history of "daft lambs" was obtained only of the flock in North Wales which had been observed by White and Rowlands [11] (1945) for some years. The origin was traced by them to the introduction in 1940 of a ram (no. 1) and by 1943 most of the ewes in this flock were daughters. In that year 2 rams were used, no. 2, distantly related to no. 1, and ram 3, who was a son. The mating of ram 2 with 9 daughters of ram 1 produced no defective progeny while the mating of ram 3 with 23 daughters of ram 1 produced 9 paralyzed lambs, but no cases of the disease were produced in the progeny by mating with 9 unrelated ewes. In 1945, 12 ewes—daughters of ram 1—were again mated with ram 3, and 4 paralyzed lambs were born. Although a detailed genetic tree was not available to us of any of the cases examined pathologically, there seemed little doubt that the condition was an inherited one as suggested by White and Rowlands [11] and that it was not due to environmental influences, nutritional defects or infective agents (unless these were inherited). This concept received some support from our study of the disease and from later observations on Canadian cases in which the genealogic tree was more fully traced, and on which comment will subsequently be made.

MATERIAL AND METHODS OF PATHOLOGIC STUDIES

Autopsies of the 11 available intact animals (9 received alive and 2 carcasses) were made in the usual manner, particular attention being paid to the nervous and muscular systems. In a few cases sections were made of all visceral organs; in all the brain and cord were fixed intact in 10 per cent neutral formaldehyde-saline solution before slicing except when portions were fixed in alcohol or formaldehyde-ammonium bromide. Preparations were thereafter made on frozen, paraffin and celloidin sections by general and special neuropathologic technics. For control purposes, material from a number of fetal and young lambs of various ages was available for study.

ANATOMIC OBSERVATIONS

Lesions were not found in the viscera, the umbilicus or the joints except in those lambs which died from some terminal illness (abscess of

the lung or pneumonia), and apart from some wasting there were no lesions in the skeletal muscles. The brain, the cord and the peripheral nerves were normal. The cerebrum seemed fully developed for lambs of the respective age groups. There was no recognizable reduction in the size of the cerebellum; this did not mean that there was no reduction of the weight, for later microscopic examination showed that there was some decrease in volume of some folia. It would, however, have been difficult to detect this with the naked eye alone unless an affected cerebellum had been measured and weighed side by side with the cerebellum of a normal lamb.

HISTOLOGIC OBSERVATIONS

There were no lesions in the viscera or in the limb muscles. From a preliminary study, sway-back, viral or bacterial infections and trauma were readily excluded. There were no cellular changes in the neurons of the motor area or of the red nucleus, no demyelination and no inflammatory lesions. The primary changes were restricted to the cerebellum.

Nature of the Cerebellar Lesions.—The initial changes were noted in some of the Purkinje cells as alterations in staining affinity, with an accompanying glial proliferation in the molecular layer. Some Purkinje cells, however, retained their normal staining capacity but showed small vacuoles mainly at their base, or the cells were swollen and ballooned by one or two enormous vacuoles; the precise basal margins of these cells were then lost and faded into the adjacent ground substance. With many Purkinje cells affected in any one folium the whole layer appeared as a fenestrated, or reticulum-like, structure; the subjacent granular layer simultaneously showed a decrease in cellular density. All these changes were seen just as readily in hematoxylin-eosin as in Nissl preparations (figs. 5 to 9).

Tinctorial changes were often much more marked; an occasional normal Purkinje cell was seen here and there between many pale-stained "ghost forms" which still retained the general outline of Purkinje cells but in which the nuclei had disappeared and the cell body had become amorphous or foamlike (fig. 8). In the most severe state of change, all cellular detail was lost, and the layer was transformed into an indistinct reticular network; the latter appearance was typically found in case 7, in which the granular layer was severely involved and showed a general poverty of all cell types. Peculiar filamentous homogeneous structures (occasionally more globose or angular, lying in retraction spaces), stained pink with eosin and yellowish with Nissl–orange G, were present deep in the granular layer; these anomalous structures showed no obvious nuclear or cytoplasmic details (fig. 9). Whether these represented aberrant Purkinje or Golgi cells which had undergone this trans-

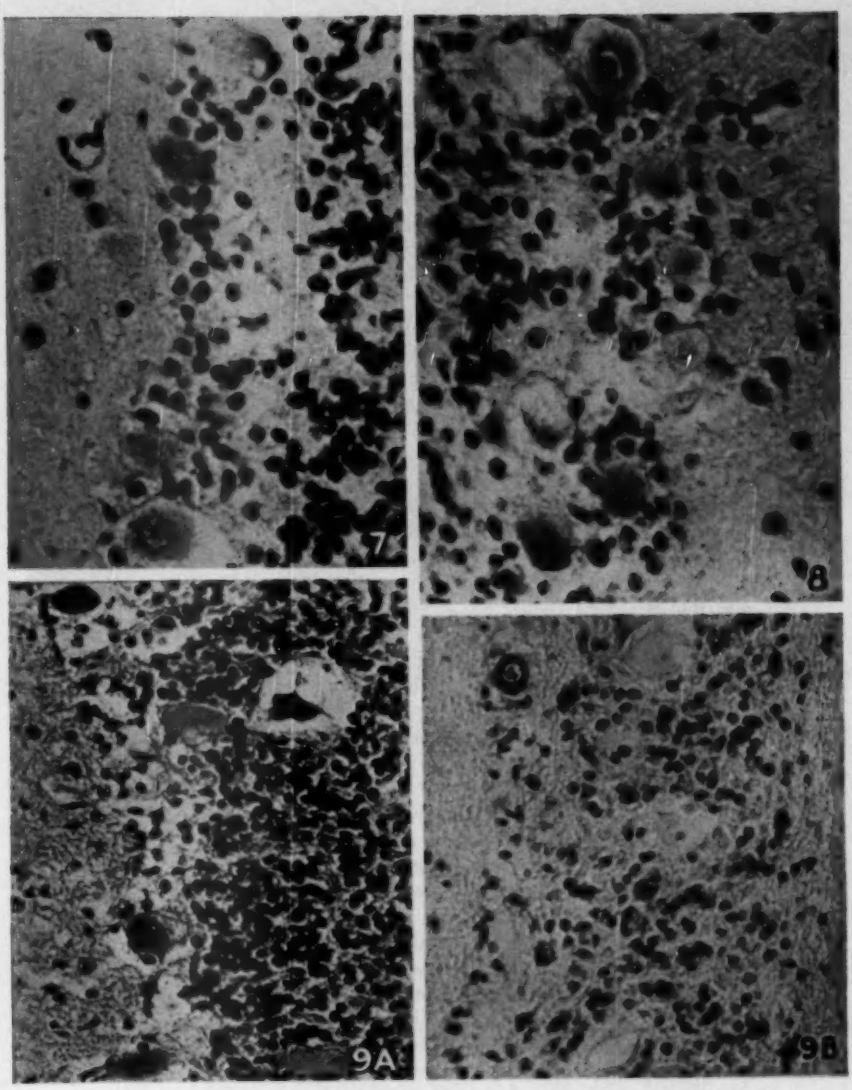

Fig. 7 (case 3).—Cerebellar atrophy in "daft lamb" kept alive for nearly a month. The Purkinje cell layer is disintegrated; a reticulum-like structure separates the molecular from the granular layer; one Purkinje cell at the top is almost completely lysed. Paraffin, Nissl; × 120.

Fig. 8 (case 9).—Cerebellar atrophy in a "daft lamb." There are advanced foamy dissolution of Purkinje cells and advanced proliferation from the Bergmann layer. Paraffin, hematoxylin and eosin; × 120.

Fig. 9 (case 9).—*A*, Purkinje cell layer, with a reticulum-like appearance and no normal surviving cells; the glial reaction is regressing, Golgi cells are involved and there are filamentous amorphous eosinophilic structures in the granular layer; one of the latter is indentated and lies in a retraction space. Paraffin, hematoxylin and eosin; × 88. *B*, another area, showing an areolar-like appearance of a wasted granular layer. Paraffin, Nissl; × 88.

formation is debatable. In many situations, however, these filamentous bodies seemed to be related to a degenerated Purkinje cell; so they might also represent some swelling (torpedo) on the corresponding axon (fig. 11). The Golgi cells were also implicated in the same type of foamy, vacuolar and lytic degeneration. An extreme degree of abnormality was seen in case 6 (fig. 9*B*), in which the whole granular layer was fenestrated with microcavities, and persisting normal Purkinje cells were present only here and there.

In Cajal preparations (fig. 10) there was great fibrillary increase arising from the proliferation of astrocytes, seen more particularly in the neighborhood of capillaries; the gliosis involved the whole of the granular layer but not the white matter or the molecular layer. "Empty baskets" and "torpedo"-thickened axons were present (fig. 11), but the latter were scarce.

In the dentate nucleus (fig. 12) lesions were fairly uniform in type; some neurons were hyperchromatic, while others showed a foamy appearance such as that noted in the Purkinje cells. Glial cells with large, clear nuclei were preserved, but the dentate nucleus was sometimes rather obscured by the relatively dense microglial proliferation—round and spindle-shaped forms; oligodendroglia was augmented in number of cells. A few of the larger neurons showed a condition of atrophic and hyperchromatic sclerosis (chronic cell lesion of Nissl) with lipoid granules in the dendrons. Comparable lesions were found in the olives of some lambs.

Remnants of the external granular layer were disintegrated and somewhat edematous, the subarachnoid space was dilated and the capillaries were engorged, but there was no cellular reaction in the perivascular spaces or in the vessels themselves.

If the general picture of all the cases studied was visualized, there appeared to be three stages identified in the process: 1. There was the initial change in the staining capacity of the Purkinje cells, which might be shrunken and hyperchromatic, or swollen and pale stained and with vacuolar degeneration pronounced at the base of the cells; Golgi cells were involved simultaneously, and there was an accompanying proliferation of neuroglia of Bergmann's layer. 2. The margins of the swollen, degenerated Purkinje cells became indistinct, and the whole cell zone had the appearance of a spongy network separating the molecular and granular layers. 3. Swollen, vacuolated Purkinje cells became very numerous, normal cells had almost completely disappeared and the entire layer was disintegrated; lipoid material was present in neurons and neuroglia but the process was not marked by severe lipoid change; a fibrillary astrocytic gliosis was present in the granular layer. These cellular changes were also repeated in the dentate nucleus; in the latter as well as in the roof nuclei the gliosis should be regarded, at least partially, as a secondary reaction to the primary cerebellar degeneration.

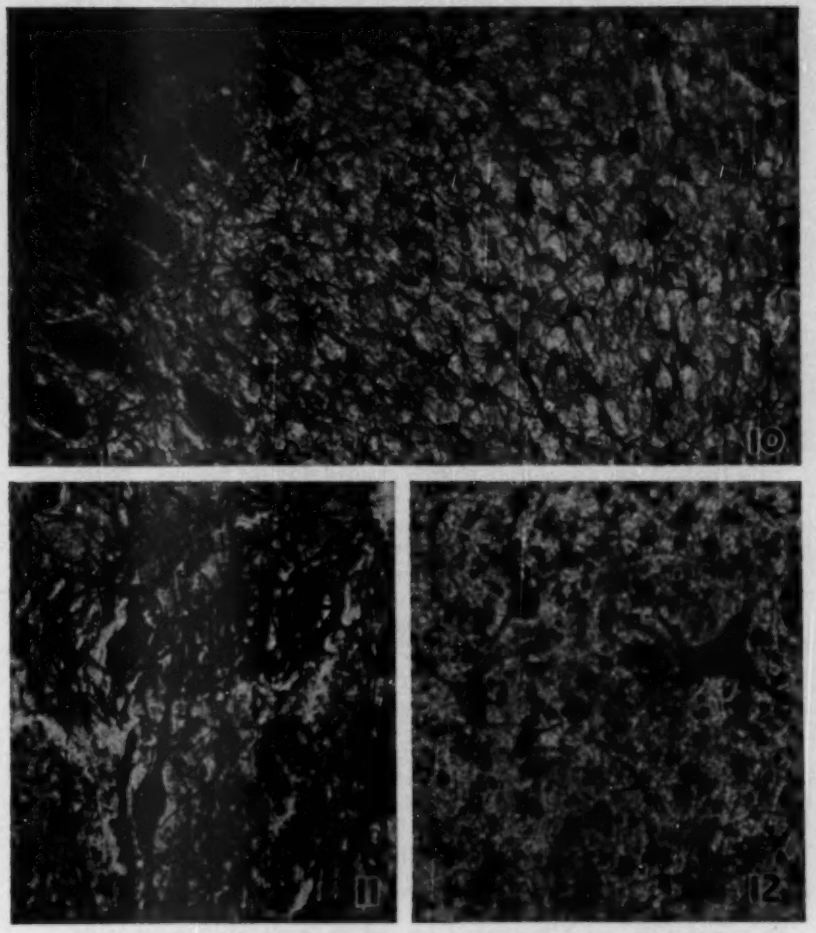

Fig. 10 (case 9).—Verticotransverse section of a folium; there is diffuse augmentation of astrocytes in the granular layer. Cajal's gold chloride-mercuric chloride; × 275.

Fig. 11 (case 9).—"Empty baskets" and one characteristic "torpedo" formation. Bielschowsky; × 88.

Fig. 12 (case 11).—Section from the dentate nucleus, showing rarefaction of ganglion cells with macrogliosis and microgliosis. Pyroxylin, cresyl violet; × 88.

This process was thus a peculiar one: It might at first seem like an edematous degenerative alteration quite different from what is known as abiotrophy or as inflammation. Further, it had a ubiquitous but nonsystematic localization on certain areas of a single part of the hindbrain; there was a complete absence of this type of lesion at higher levels such as the optic tracts or the cerebral cortex.

Distribution of the Cerebellar Lesions.—The changes were precise and constant in their quality, but their localization was variable from case to case, at least in some of the details. In all cases the median lobe was most severely affected and principally in the posterior and anterior parts rather than in the middle portion. In 2 cases in which the lingula was affected, the lesions were also most severe in the median lobe. Intensity of damage was next most apparent in the ansiform and paramedian lobes and in the flocculus and the paraflocculus, but in 1 case the ansiform and paramedian lobes were nearly normal (case 5). In 1 case (no. 2) the lingula showed no lesions. In the lambs with less severe damage (e. g., 7 and 8) the anterior and posterior parts of the median lobe were most involved. The brunt of the degeneration was roughly comparable in the ansiform and the paramedian lobe, but, in contrast, the paraflocculus was always more severely changed than the flocculus. In the white matter the central nuclei showed lesions much less regularly and always the dentate more than the roof nuclei; there were no lesions in the central nuclei in 7 cases; in another case it was impossible to determine whether there were lesions; the nuclei were severely affected in 2 cases (1 and 6) and moderately so in the remaining 3.

In some cases there were various lesions which were regarded as coincidental and of little importance, viz.: slight lymphocytic infiltrations in the choroid plexus and lateral recess (case 5), perivascular infiltrations in the interpeduncular regions and stasis of blood in the white matter of the frontal lobe (case 8), small foci of necrosis in the anterior horn of the cervical cord (case 11) and slight hydrocephalus (case 12). Most of these might have been related to some agonal infection and clearly could have had little connection with the essentially degenerative process in the cerebellum.

PROTOCOLS

Case 1 (Scotland).—Severe changes occurred in Purkinje cells, particularly in the anterior and posterior parts of the median lobe and the flocculus and the paraflocculus, less in the ansiform lobe and the lingula. Very severe lesions occurred in the dentate nucleus; minor damage, in the roof nuclei. No changes were found in other parts of the brain.

Case 2 (North Wales).—The lamb was 1 to 2 days old, dead on arrival and in a poor state of preservation. Very severe lesions were noted, more extreme than those in case 1, especially in the median lobe and the flocculus; less in the ansiform lobe. In the dentate nucleus there was some preservation of cells. The remainder of the brain was normal.

CASE 3 (North Wales).—The lamb was received alive, 1 to 2 days old, and was kept alive nearly a month by artificial feeding; it was unable to walk; its paralysis did not become progressively worse; death was caused by multiple pulmonary abscesses. The medulla, the pons and the cerebrum showed no lesions; the cerebellum had lesions of equal severity in all parts but less so in the flocculus; lesions of the dentate nucleus remained undetermined.

CASE 4 (Yorkshire).—The lamb was 2 to 5 days old. It was kept alive for a few days before being killed. The lesions of the cerebellar cortex were uniform but in initial stages. Many Purkinje cells were of normal appearance; others showed basal vacuolation. There were some filamentous globular structures in the granular layer, with some "falling out" in sections, which produced spherical or oval cavities. The roof nuclei and the dentate nucleus showed minor lesions and discrete glial reaction: in some areas Bielschowsky methods showed beading and swelling of axons and true torpedo formation. The pons and the medulla were normal.

CASE 5.—The lamb was the companion of the lamb in case 4. It was kept alive for a few days before killing and autopsy. There were small calcareous bodies in the frontal pole, some stasis in vessels of the cortex and mild lymphocytic infiltration in the choroid plexus and the lateral recess. The lesions of the dentate nucleus showed cellular rarefaction and diffuse glial response. Vacuolar degeneration was seen in Purkinje cells, particularly in the median lobe and the paraflocculus. The gray matter was intact. Occasional glial nodules were found in the white matter. Pons, medulla, cord and peripheral nerves revealed no lesions.

CASE 6 (Wales).—The lamb was 6 days old and alive when received but died shortly afterward, of bronchopneumonia. It had been unable to rise or walk (fig. 6*A*). No lesions were found in cerebrum, peduncles, cord or peripheral nerves. Lesions were present in all parts of the cerebellum except the posterior part of the median lobe; the glial proliferation was typical in this case. Purkinje cells were severely damaged and the granular layer thinned out; ballooning was seen with vacuolation of Purkinje cells; there was microcavitation in the granular layer. Some cellular elements were preserved in the roof nuclei but not in the dentate nucleus.

CASE 7 (Wales).—The lamb, 1 to 2 days old, was received in poor condition, weak but alive, and was killed immediately. With the exception of the cerebellum the nervous system was normal. Moderate perivascular infiltration was noted in the centrum ovale and in the leptomeninx. There were severe lesions in the median lobe—posterior and anterior parts—and somewhat less, in the ansiform lobe. The flocculus and the paraflocculus were little affected. There were characteristic degenerative changes in the Purkinje cells and in the granular layer. The dentate nucleus and the roof nuclei were well preserved and showed only moderate gliosis.

CASE 8 (Wales).—The lamb was 4 days old. It was kept alive until 16 days of age, showing no improvement and becoming progressively weaker. In the white matter of the frontal cortex there was some vascular stasis with edema. Some mild extra-adventitial cell reaction and lymphocytic infiltrations were seen in the interpeduncular region. The lesions of the cerebellum were most severe in the posterior and anterior parts of the median lobe; in some folia above the fourth ventricle they were very advanced, less marked in the paramedian lobe and the paraflocculus. The dentate nucleus and the roof nuclei were normal.

CASE 9 (Northumberland).—The lamb was received alive, 1 to 3 days old, and able to walk with difficulty (fig. 6*B* and *C*). No lesions were found in the cerebrum.

Characteristic initial lesions were present in the posterior part of the median lobe, the ansiform lobe and to a lesser degree in the paraflocculus. Rarefaction of Purkinje cells and of the granular layer were not far advanced. The dentate nucleus was normal.

Case 10 (Wales).—Only the carcass of a newborn lamb was received, and it was in a poor state of preservation. Cerebrum, brain stem and cord were normal. In the cerebellum were extremely advanced lesions involving the totality of the lobes with the exception of the anterior part of the median lobe; they were most noticeable in the posterior part of the median lobe, the lingula, the flocculus and the paraflocculus and much less severe, although still impressive, in the ansiform lobe. The dentate nucleus and the roof nuclei were intact.

Case 11 (Northumberland).—The lamb was 2 days old and unable to rise. Only the brain was sent. The cerebrum was normal. In the cord there was a small focus of softening in the cervical region in the anterior horns. Typical lesions were found in the cerebellum, mostly in the median lobe, the paraflocculus and the flocculus and a little less notable in the ansiform lobe. The dentate nucleus and the roof nuclei were intact.

Case 12 (North Wales).—A newborn lamb was sent alive. It was unable to rise. Autopsy revealed a slight degree of hydrocephalus and dilatation of the central canal. Typical lesions were encountered in the anterior and posterior parts of the median lobe, less typical in the folia near the primary fissure and less typical still in the flocculus, the paraflocculus and the ansiform lobe. The lesions were of about the same degree of severity in these parts. The central nuclei were normal.

Case 13 (Northumberland).—The lamb was a few days old. Only the nervous system was examined. The cerebrum, the brain stem, the cord and the spinal ganglion were normal. Typical lesions were observed in the cerebellum, more severe in the paraflocculus than in other parts. The roof nuclei were intact.

III. CORTICAL CEREBELLAR ATROPHY OF CORRIEDALE LAMBS IN ALBERTA, CANADA

Attention was drawn by Innes and co-workers [12] (1949) to the paper by Rasmussen [13] (1943), who described "a new hereditary lethal for sheep with nervous incoordination and paralysis." The disorder was first seen by him in 1942, but no pathologic examinations were made. From the brief clinical descriptions given and from the photographs of affected lambs it was clear there was some similarity to the "daft lambs" seen in Britain. The brains and cords (fixed in formaldehyde-saline solution) of 3 Canadian lambs presenting this disease, collected in the lambing season of 1949, were received by one of us (J. R. M. I.). No macroscopic changes were present in the brains; sections were prepared from these organs after paraffin and celloidin embedding at the Armed Forces Institute of Pathology, Washington, D. C., through the courtesy of Dr. Webb Haymaker, Registry of Neuropathology.

In each of these 3 Canadian lambs, microscopic lesions identical with those described for "daft lambs" were present in the cerebellum. The changes were restricted to that part, and while there was some quantitative variation, they were qualitatively precise (fig. 13 *A*, *B* and *C*).

13. Rasmussen, K.: Scient. Agric. **25**:482, 1943.

IV. PROGRESSIVE CEREBELLAR ABIOTROPHY OF A LAMB

A lamb, aged 10 to 14 days, was examined by one of us (J. R. M. I.) in 1935 during the sway-back investigations then being made. The animal was unable to stand and came from a farm in Radnorshire, South Wales, with the history that it was the only one affected with a disease which was quite unlike that of

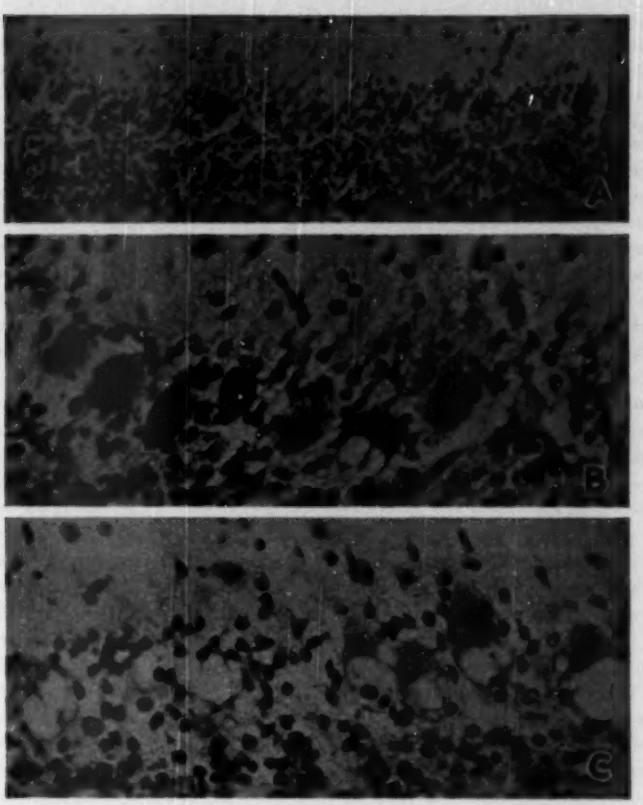

Fig. 13.—Cerebellar atrophy in a lamb from Canada. *A*, low power view, showing few normal persisting Purkinje cells; some are shrunken and pyknotic and others vacuolated; there is slight thinning of the granular layer. *B*, higher power view, showing two pyknotic forms, one with two vacuoles at the base. *C*, high power (× 185) view, showing vacuolar change in all Purkinje cells in the field. Paraffin, hematoxylin and eosin.

sway-back. No lesions of sway-back were found. Weigert-Pal celloidin sections of cerebrum and midbrain, Marchi-Busch preparations of medulla and cord,

and hematoxylin-eosin and Nissl methylene blue-stained sections of various parts, including the cerebellum, had been filed and were available for study when the problem of "daft lambs" arose.

PATHOLOGY

Verticotransverse sections of the cerebrum through the level of the anterior hypothalamic regions showed no changes. Cortex, white matter, hippocampus, basal ganglions, optic tracts, central nucleus of Luys and substantia nigra, tuber and chiasma were all intact. The cerebral peduncles at the caudal level of the oculomotor nuclei, the corpora quadrigemina and the pyramids showed no lesions; the red nucleus unfortunately had been missed when the block of tissue was cut. The cerebellum showed unmistakable reduction in volume of the lateral hemispheres (the sections which were used for illustration had been cut from the posterior part). Under the low power, atrophy affected all layers and was most advanced in the flocculus, the parafloccculus and the paramedian lobe. The folia bordering on the primary fissure and the posterior part of the median lobe showed some atrophy, while the folia of the lobus simplex, visible in the median line, showed changes comparable to that seen in the parafloccculus. There were no lesions of the cerebellar white matter. Only part of the dentate nucleus was found and the nerve cells were normal; the medulla was normal, but unfortunately the olives were not present in the sections kept. There was some augmentation of glia in both the cervical and the thoracic portion of the cord, in the posterior columns and around the column of Goll, but no degeneration was noted in Marchi-Busch or Weigert-Pal preparations.

A striking feature of the cerebellar changes was the apparent phases of the process in different parts. In the anterior part of the median lobe the Purkinje cells were more or less normal, although they were commonly displaced into the molecular and granular layer (heterotopia) (fig. 14*A*). Neuroblasts were often present in groups of three or four, and there was some augmentation of oligodendroglia. In other parts Purkinje cells were more fragmented; the whole layer was disintegrated, and neuroblasts were present. In still other areas most of the Purkinje cells had disappeared and only neuroblasts and a few Golgi cells were present (fig. 14*B*), and in the most severely affected folia all such normal structural elements had vanished (fig. 14*C*). The gray matter then showed as a reticular tissue with numerous glial cells which might have represented fibrillary gliosis; fresh fixed tissue or paraffin blocks were not, however, available to make the necessary selective technics.

COMMENT

The cerebellar disorders described in lambs have not been previously reported. (See the records of lethal and sublethal inherited conditions

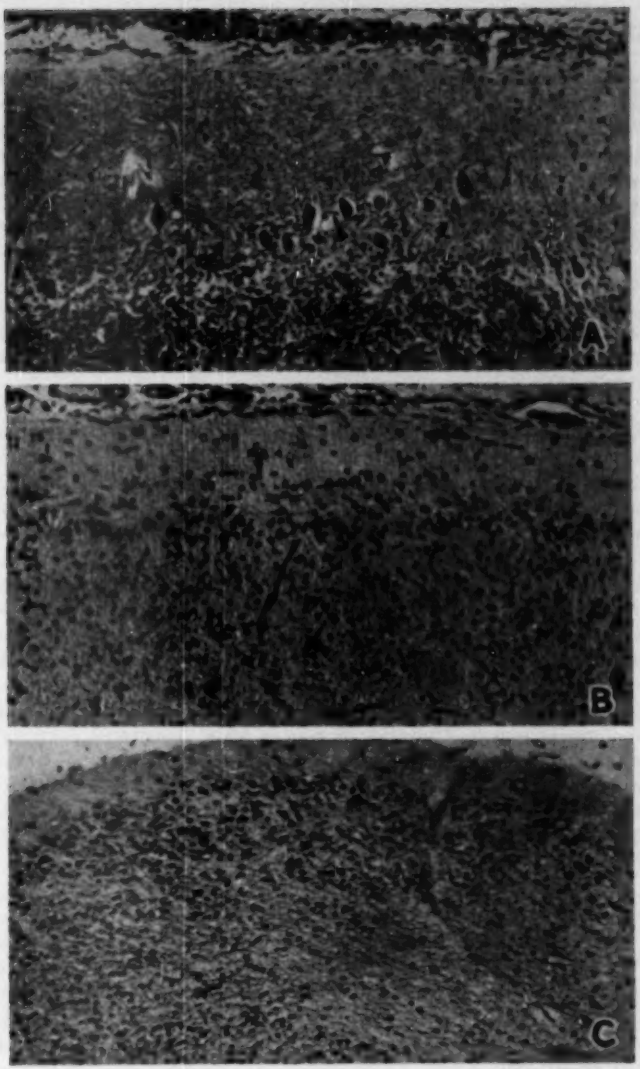

Fig. 14.—Progressive cerebellar abiotrophy in a 10 to 14 day old lamb, suspected to have sway-back. *A*, anterior portion of the median lobe of the cerebellum. Some normal Purkinje cells are preserved, but many are displaced into upper parts of the molecular layer; there is some glial reaction. Hematoxylin and eosin; × 88. *B*, flocculus. There is complete disappearance of Purkinje cells, with replacement gliosis; the granular layer has undergone great wasting, but many Golgi cells have persisted. *C*, paramedian lobe. An extreme degree of atrophy has affected all levels of the granular layer and Purkinje cells. Paraffin, hematoxylin and eosin; × 88.

of farm animals by Eaton [14] [1937], Lerner [15] [1944], Hutt [16] [1946] and Günther Just [17] [1939-1940]). Forms of cerebellar disease have, however, been described in other species, such as the cat, the dog, the Hereford calf and the monkey, references to which can be found in the papers of Brouwer [18a] (1934), Finley [19] (1935, Innes, Russell and Wilsdon [20] (1940), van Bogaert and Dallemagne [21] (1943), Schut [22] (1946) and the monograph on comparative neuropathology by Scherer [23] (1943). The cerebellar conditions described by these and other authors might be compared with certain human forms of cerebellar disease, heredofamilial examples of which are well recognized; although the latter may differ in their precise localization and in the nature of the process they might all be mutually related on some, as yet, undetermined genetic basis.

From the history of "daft lambs" originally observed by White and Rowlands (1945), meager though it may be, the condition could be included in the category of inherited disorders. Experimental brother–half-sister matings in 1943 and 1945, resulting in a high incidence of paralyzed progeny as compared with no defective lambs from matings of unrelated rams and ewes, appear to be sufficient proof of this contention. Cerebellar atrophy in lambs, therefore, is another disease to be added to the genetic disorders of animals which are relics of attempts by breeders to fix certain characteristics in farm stock by inbreeding and line breeding—relics which crop up in the form of certain lethal and sublethal diseases. The list of the latter established in the literature is now lengthy (see Eaton [14] and Lerner [15]); some of the better known defects include hairless and amputated calves, spina bifida, "lethal contractures of muscles," absence of eyes in foals, "congenital paralysis" in pigs, achondroplasia (bulldog calves), various congenital spasms and cerebellar atrophy in Hereford calves. Unfortunately, even scanty details of the breeding history of "daft lambs" from other districts in Britain were unobtainable. The identification of the disease in Corriedale

14. Eaton, O. N.: J. Hered. **28**:320, 1937.

15. Lerner, I. M.: J. Hered. **35**:219, 1944.

16. Hutt, F. B.: Cornell Vet. **36**:180, 1946.

17. Just, G.: Erbleiden des Zentralnervensystem bei Säugetiere, in Handbuch der Erbbiologie des Menschens, Berlin, Julius Springer, 1939, vol. 5.

18. Brouwer, B.: (*a*) Psychiat. en neurol. bl. [**38**]:352, 1934; (*b*) Fol. neuropath. eston. **15**:16, 236, 1936.

19. Finley, K. H.: Proc. Soc. Med., Amsterdam **28**:1, 1935.

20. Innes, J. R. M.; Russell, D. S., and Wilsdon, A. J. (1940): J. Path. & Bact. **50**:455, 1940.

21. van Bogaert, L., and Dallemagne, M. J.: J. belge de neurol. et de psychiat. **43**:271, 1943.

22. Schut, J. W.: J. Neuropath. & Exper. Neurol. **5**:77, 1946.

23. Scherer, H. J.: Vergleichende Pathologie des Nervensystems der Säugtiere: Ein Versuch, Leipzig, Georg Thieme, 1943.

lambs in Canada is important, for through the cooperation of Dr. Rasmussen and Mr. W. A. MacNaughton (Alberta, Canada) a detailed genealogic tree was prepared of the 3 cases in Corriedales examined in 1949 (fig. 15).

All lambs identified in the chart other than the 3 in cases 1 to 3 were normal for many years. The 3 sires 191 U, 147 P and C 258 were mentioned in the record of a "new hereditary lethal for sheep" by Rasmussen [18] (1943); ram 191 U was used for several years in experimental matings; when he was bred to ewes sired by his sire, 147 P, or grandsire, C 258, several paralyzed lambs were born. This defect

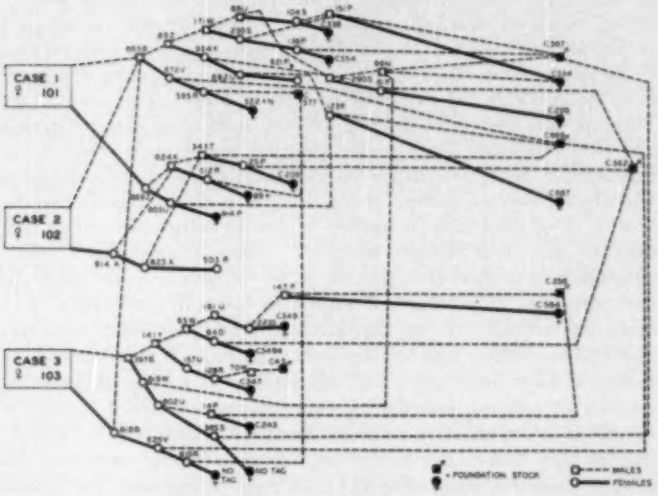

Fig. 15.—Ancestry of 3 "daft" Corriedale lambs, 1949.

was thought then to be restricted to this male line but the 3 cases of 1949 (all daughters or granddaughters of 865 B) can also be traced back to ram C 567—one of the foundation males of the flock; the gene is evidently distributed throughout the flock. Rasmussen concluded that the defect was conditioned by a recessive gene but was not necessarily a simple case of homozygous recessive. The fact that this cerebellar atrophy has been observed in newborn lambs in situations as far apart as Canada and Britain suggests that it might occur wherever sheep of the initial stock for Canadian Corriedales, or pure Corriedales, have been used; the disease may have entirely escaped observation or may have been regarded as a "clinical disorder."

The fact that "daft lambs" showed signs at birth may not be of exceptional moment, because the structural development of the ovine nervous system at birth has a remarkable precocious maturity (Romanes [18]). The newborn lamb can perform all its motor functions shortly after birth, and this may be related to the advanced state of myelination.

Careful investigation might elucidate the formal genesis of the disease more easily than the mechanism underlying the nature of the lesion. The genetic and pathologic data are of no help in this direction, and it might be disputable in which category of cerebellar disease "daft lambs" can be included. It cannot be regarded as a dysplasia, for there was no sign of any other microscopic or macroscopic malformation—heterotopias, anomalies of cellular positions or of forms of cells—in any part of the nervous system. The pathologic process was not a uniform one, in the sense that it varied from case to case without showing any specificity for attacking any particular neurons or fibers. The fact that the Purkinje cells were most severely affected in all cases does not permit any valid conclusion being drawn regarding the systemic effect of the degeneration, for these cells are the most fragile of all in toxic disorders affecting the nervous system. It is possible to exclude a concept that the process is an abiotrophy as applied by human neurologists when considering the systematized atrophies (olivopontocerebellar types), although it is in this sense that the name we have proposed might be most criticized. It is feasible to regard the lesion in "daft lambs" as a toxic degeneration affecting the cerebellar cortex, for the process was essentially a swelling with ultimate lysis of neurons. It would seem to be evoked at some late stage of fetal development as a subacute metabolic disorder of neurons, initiated by some unknown inherited cause. In support of this it might be contended that the Purkinje cells had already reached their proper destination and had subsequently undergone the atrophic processes described or had completely disappeared. The process might thus be compared to that of premature cellular senesence which is postulated by some workers to form the substratum of abiotrophies of man.

The conditions in kittens described by Finley [19] and Brouwer [15] are true examples of olivopontocerebellar hypoplasia. The familial cerebellar disease of Hereford calves recorded by Innes, Russell and Wilsdon [20] showed gross atrophy of the cerebellar cortex, and it was believed that this process represented a combination of arrested development and degeneration. The cortical cerebellar atrophy in a monkey described by van Bogaert and Dallemagne [21] was a systematized lesion of the Purkinje cell layer affecting the whole organ. The lesions in all these forms are quite unlike those of "daft lambs," which are characterized by specific forms of cellular degeneration.

The process described in part IV, which occurred in 1 lamb, 14 days old, manifestly represents a progressive cerebellar atrophy, or an abiotrophy, involving all cortical elements with varying degrees of severity in different parts and with replacement gliosis, and is thus quite analogous to some atrophies described in man. Heterotopia of the Purkinje cells was quite prominent in those areas where there were still remnants of the molecular and granular layers (fig. 14*A*) and constituted an important difference as compared with the relatively rare occurrence of dislocation of the same cells in "daft lambs." This kind of lesion has been the subject of comment on the part of many authors, and the exact mechanism of its development is unknown. Norman [24] (1940) gave much attention to it in his description of peculiar human cases of primary degeneration of the granular layer in early life, and some of his contentions might be applied here. An atrophic process might initiate irregularities of position of the Purkinje cells, but it cannot be solely responsible. If this were not so, then many intermediate types of lesion with cellular dislocations of varying degrees would have been found in the atrophic process in the "daft lamb series." The time of onset of atrophy, which in the case of the lambs was *in utero,* might be much more important in interfering with normal migration of Purkinje cells. In this particular case of abiotrophy in a lamb it would seem that there was a clear arrest of migration as opposed to the characteristic vacuolar and lytic disintegration of Purkinje and Golgi cells, which had already reached their proper destination, in the typical cases of "daft lambs." In some ways the course of the case is also different from that of "daft lambs" for in 1 lamb of the latter variety which survived a month the cerebellar lesions were in effect no more severe than in other lambs 1 or 2 days old.

From what is known about cerebellar function in the lower animals the lesions in "daft lambs" can be regarded as responsible for the signs noted at birth, namely, hypotonia, asthenia, opisthotonos, sometimes coarse tremors and incoordination. The violent convulsive movements which were seen in the Hereford calves were not noted in the lambs, nor was nystagmus observed.

In the introduction it was mentioned how "paralytic entities" of lambs can be easily confused clinically in the field, and examples were cited. In 2 of the 15 reputed "daft lambs" submitted for examination, pathologic features were present in the nervous system which indicated they could not be placed in the same category as the others. In the appendix these 2 lambs are described, as they may be of general interest or of particular value to workers who may desire to study other nervous diseases of lambs.

24. Norman, R. M.: Brain **63**:365, 1940.

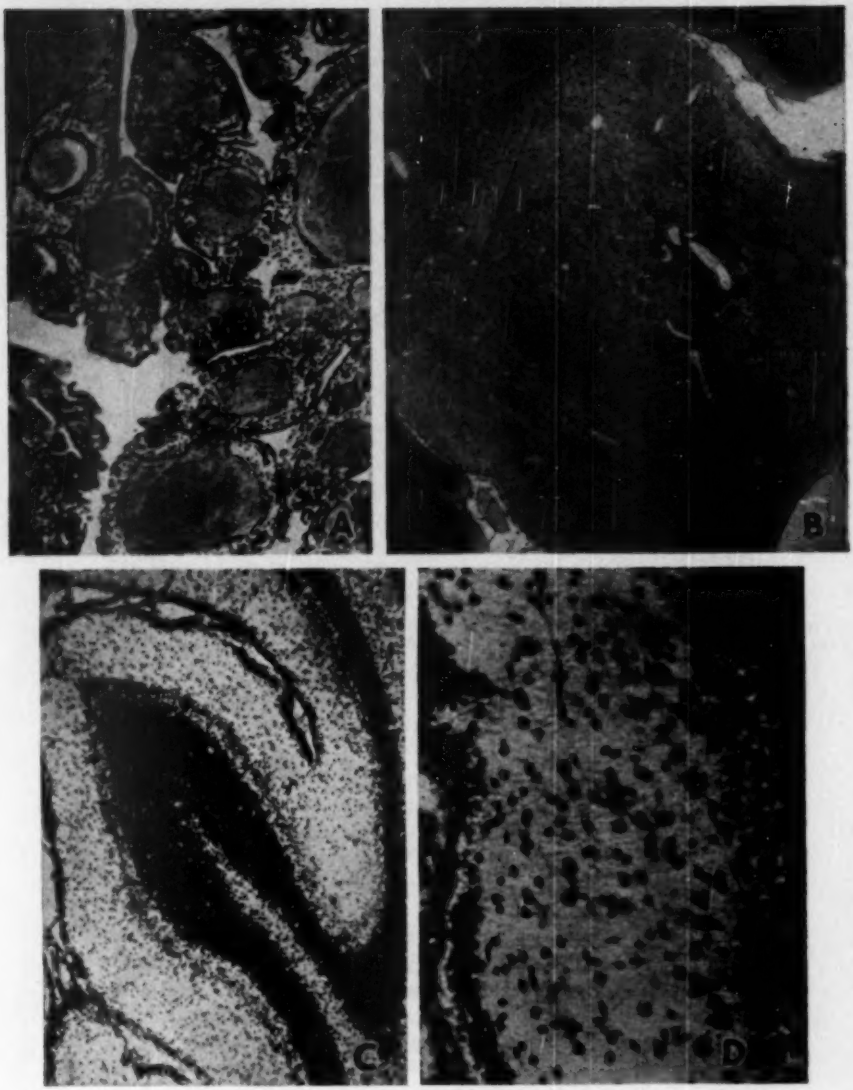

Fig. 16.—Angiomatosis with cerebellar atrophy in a 2 day old lamb. *A*, large dilated veins in the choroid plexus (× 20). *B*, newly formed veins in the pulvinar (× 20). *C*, wasting of the granular layer in the cerebellum, with disappearance of Purkinje cells (× 88). *D*, neuroblasts in the molecular layer, some glial reaction, absence of Purkinje cells and some lymphocytic infiltration in subpial zones on the left (× 120). Pyroxylin, cresyl violet.

APPENDIX

ANGIOMATOSIS OF THE NERVOUS SYSTEM—A MALFORMATION—COMBINED WITH CEREBELLAR ATROPHY IN A LAMB

The lamb died two days after birth; it was stated to have been born without a tail and with some scoliosis. Only the brain and cord were available for examination.

Pathology.—In the cerebellum (fig. 16 *C* and *D*), particularly in the median lobe, flocculus and ansiform lobe, there were poverty and degeneration of Purkinje cells and a wasting of the gray matter accompanied with regressive change in the glia; the cells of the latter were rounded, swollen, with pyknotic and shrunken nuclei. Persisting Purkinje cells were mainly swollen and ballooned and contained vacuoles; many Golgi cells showed a similar change. In parts where the cerebellum was less damaged the molecular layer was traversed by numerous vessels—tributaries of the leptomeningeal veins—and numerous globular cells were present which might have been microglia with regressive changes or neuroblasts.

Large dilated veins of a diameter two to three times that of the normal veins were also present in the leptomeninges. They formed clusters at the depths of the fissures and were prolonged in waterspout fashion over the surface of the convolutions. Arteries and arterioles were normal, and there was no thrombosis, malformation of venous sinuses or cellular meningeal reaction.

Dilated, tortuous veins were scattered widely throughout the nervous system, and the choroid plexus was involved (fig. 16 *A*). The "angiomatosis" or "phlebectasis" (fig. 16 *B*) was most marked in the frontoparietal regions, less in the temporal and occipital poles; the hippocampus was not spared, but there was little involvement of the corpus callosum and trigone. Glial clusters, associated with myelogenesis, were very marked in the white matter of the temporal lobes, the angular gyrus, the occipital lobes and optic radiations. Residual neuroblasts were not more prominent than usual. Sections through the caudate nucleus, putamen and thalamic fissure showed numerous venous dilatations but none in the mamillary body, the red nucleus or the pulvinar. The choroid plexus of the lateral ventricles was less altered, but the sphenoid sinus contained a huge cluster of veins. Small venous malformations were numerous in the gray and white matter of the temporal lobe anterior to the cerebral peduncles and were disposed in rather a radial manner; they were also rather prominent in the corpora quadrigemina, geniculate bodies and pineal gland. The cerebellum as a whole was not involved in the vascular malformation, but the white matter around the dentate nucleus and roof nuclei contained many tortuous venous structures while there were none in the middle cerebellar peduncles or medulla and only a few in the pons. In the thoracolumbar portion of the cord there was dilatation of the central canal, which contained detritus, with an accompanying gliosis around; where the dilatation was maximal, venous malformations in gray and white commissures surrounded the canal.

Comment.—This case represents an angiomatosis-like condition of the meninges and nervous substance extending in different degrees through the entire axis. It was most marked in the upper levels of the cerebrum, faded out in the cerebellum and brain stem and appeared again in the lower parts of the cord; it also involved the choroid plexuses and pineal body; there was also hydromyelia. Cerebellar lesions were present almost identical with those of "daft lambs"; hence it would seem that this was a case of vascular malformation on which was superimposed cerebellar degeneration; the former could not explain the presence of neuroblasts and glial proliferation at the level of the Purkinje cell layer. The lamb showed

absence of tail and scoliosis. The complex thus might be compared with certain poorly understood diseases of man which show syringomyelia with angiomatosis. It is possible to consider that in this lamb the vascular malformation favored the initiation of the cerebellar disease, for it is known that in man angiomatosis predisposes to other cerebral conditions.

SUBACUTE MENINGOENCEPHALITIS OF THE MESENCEPHALOCEREBELLAR LEVEL IN A LAMB

One of the 15 animals submitted for examination as a suspected "daft lamb" was a 2 day old lamb from North Wales; examination was limited to the nervous system.

Pathology.—The essential changes were diffuse, and focal, glial infiltrations related to vessels (fig. 17 *A*), endothelial proliferation and extra-adventitial infiltrations. Occasionally neurons showed signs of vacuolar dissolution, but in no part of the nervous system were these clearly the primary lesion and there was no sign of neuronophagia. Although endothelial proliferation was outstanding, there was no thrombosis. In the cerebellar cortex there was some multiplication of newly formed capillaries and in the leptomeninges a mild degree of lymphocytic infiltration. The neuroglial reaction mainly concerned microglia cells and astrocytes with some fibrillary increase. Lesions of myelin were doubtful.

The severest lesions of this type were present in the midbrain and in the cerebellum and in the former as diffuse and perivascular glial and lymphocytic collections in the oculomotor and red nuclei. There was some atrophy of the whole cerebellar cortex visible to the naked eye; it was marked in the folia of the more central part of the median lobe, the flocculus and the paraflocculus but not in the anterior part of the median lobe or the ansiform lobe. One face of a folium perhaps showed severe atrophy while the other one might be almost intact; the atrophic process affected both Purkinje cells and the granular layer (fig. 17 *B* and *C*). There was some fine glial proliferation of the molecular layer of the paraflocculus. The dentate nucleus also showed perivascular reaction which extended into the adjacent white matter, in which there were also small glial clusters. The pons was not entirely free from lesions; the facial nuclei were intact but in contrast the stria medullaris, the medial longitudinal bundle showed some focal infiltrations with some degree of spongy state. In the focal infiltrations glial elements predominated over cells of hematogenous origin and, although mainly microglia cells, astrocytes were also present; focal nodules were sometimes contiguous with perivascular infiltrations. In the medulla similar foci were present in the nucleus of the twelfth nerve, in the reticular substance and in the olives, while in the cord they were mainly in the lateral columns, together with some microcavitation. The periependymal regions and the posterior horns were free from changes.

Comment.—The pathologic process in this lamb appears to have been a patchy inflammatory one which affected quite irregularly the red nucleus, the cerebellar cortex, the dentate nucleus, the reticular substance, the olives, the hypoglossal nuclei and the anterolateral horns of the cord. There was an absence of any selective degenerative process affecting the Purkinje cells as in "daft lambs." Other points of difference included the focal proliferation of microglia, the fibrillary increase in the molecular layer and the focal and perivascular infiltrations in the cerebellar white matter and central nuclei; the process was also widely spread throughout the gray matter of the midbrain, pons, medulla and spinal cord. As the examination was limited to the nervous system, one cannot exclude the possibility

that this process was simply an example of secondary effects on the nervous system of a septicemia arising from some infection elsewhere in the body. Such lesions with predominating glial participation are seen in a variety of systemic intoxications and infections of man and other animals. Unfortunately, no details were

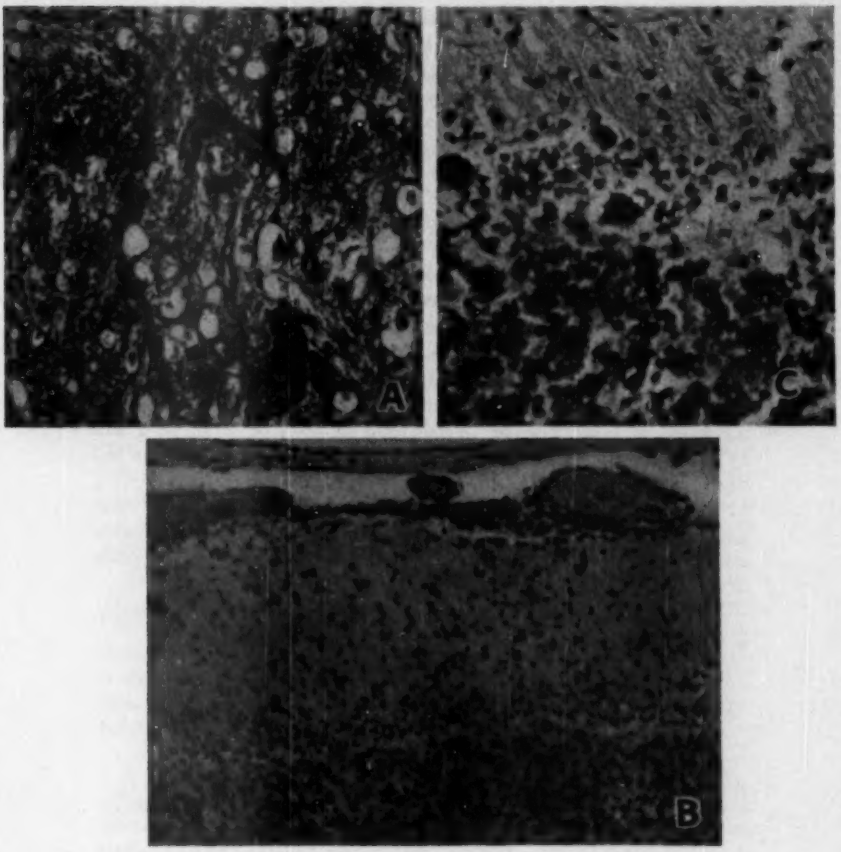

Fig. 17.—Meningoencephalitis in a suspected "daft lamb" 2 days old. *A*, pons, showing focal lymphocytic and glial proliferation (× 55). *B*, vermis, showing disappearance of Purkinje cells, microglial and macroglial infiltrations in the molecular layer and perivenous lymphocytic infiltrations in the pia (× 55). *C*, vermis, showing shrinkage of persisting Purkinje cells and disappearance in adjacent parts (× 88). Paraffin, hematoxylin and eosin.

obtainable to clarify this point, but young lambs suffer from various generalized infections acquired at, or shortly after, birth. It seems unlikely that the lesions

were due to a neurotropic virus; the age of the lamb (2 days) plus the lack of acute selective changes in neurons might obviate this possibility, while the cerebellar lesions are unlike those seen in louping-ill of older sheep. Toxoplasmosis was also excluded.

SUMMARY

Brief comments with illustrations are made on normal ovine neuroanatomy with particular reference to the cerebellum.

A disease of newborn lambs (of different breeds) with signs of incoordination and hypotonia was identified as occurring in Britain, where affected animals are known in local regions as "daft lambs."

The primary lesions were restricted to the cerebellum and consisted essentially in swelling, vacuolation and lysis of the Purkinje cells and, to a less extent, the Golgi cells; glial reaction, initiated in the granular and Bergmann layer, was a consequent feature; corresponding neuronal and glial changes were present in many cases in the dentate and roof nuclei. The condition was concluded to be a form of cortical cerebellar atrophy, and some evidence was given to show that it was inherited.

An identical cerebellar condition was later established to occur in Corriedale lambs in Canada; more data were available to indicate that the disease was inherited and due to a recessive gene.

The causal genesis is conjectural; it was suggested that it might be a metabolic, or toxic, disorder of cerebellar neurons occurring at a time in fetal development of the lamb when migration of Purkinje cells was nearly complete.

Other "paralytic entities" have been confused clinically with "daft lambs," and 2 such cases are described: (*a*) angiomatosis with cerebellar atrophy and (*b*) a peculiar meningoencephalitis of the mesencephalon of uncertain etiologic origin.

A case of true progressive cortical cerebellar atrophy, or abiotrophy, in a lamb is described.

SUBCUTANEOUS BERYLLIUM GRANULOMA

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AND
CYRUS C. ERICKSON, M.D.
DURHAM, N. C.

WHILE the pulmonary lesions of beryllium poisoning have received widespread consideration (see Tabershaw, Dustan and Goldwater [1] for a complete bibliography), the cutaneous manifestations have been almost completely overlooked. The lesions of the skin are of four types,[2] namely, contact dermatitis, ulcers, lesions arising spontaneously in patients with chronic pulmonary beryllium granulomatosis, and subcutaneous granuloma. The last-named lesion, the subcutaneous beryllium granuloma, was first described by Grier, Nash and Freiman [2]; they reported 3 cases in which the lesions followed, and were associated with, cutting the skin on fluorescent lamps. The only other reported instances are those of Nichol and Dominguez,[3] who described 2 more cases. In every instance there was a clearcut history of the skin's having been lacerated by fluorescent tubes, of slow and improper healing of the wound, intermittent pain, swelling and tenderness, and, often, spontaneous ulceration. In all cases cited, an apparent cure resulted from surgical excision of the involved areas.

Recently, an example of this condition came under our observation. The paucity of reported instances, as well as the fact that this lesion bore a striking histologic similarity to Boeck's sarcoid of the Darier-Roussy type, makes this case worthy of note and of particular interest in that it emphasizes the occurrence of this morphologic type of granulomatous reaction as a result of an injury caused by this specific agent, beryllium.

REPORT OF A CASE

A 12 year old white boy was admitted to Duke Hospital (Unit C 67089) complaining of a tender, thick, callus-like palmar scar. Two years previously he had picked up a cracked fluorescent lighting unit, which fragmented in his hand, causing a 2.5 cm. laceration over the thenar eminence. This failed to heal properly, and the area continued to be tender and annoying.

From the Department of Pathology, Duke University School of Medicine.

1. Tabershaw, I. R.; Dustan, C., and Goldwater, L. J.: J. Indust. Hyg. & Toxicol. **31**:227, 1949.

2. Grier, R. S.; Nash, P., and Freiman, D. G.: J. Indust. Hyg. & Toxicol. **30**:228, 1948.

3. Nichol, A. D., and Dominguez, R.: J. A. M. A. **140**:855, 1949.

Physical examination confirmed the foregoing complaint but gave otherwise negative results. The chest was clear. The regional lymph nodes were not enlarged. Laboratory data indicated normal conditions.

The palmar scar was widely excised, and the patient made an uneventful recovery.

The surgical specimen, a rectangle of skin and subcutaneous tissue 1.5 by 1.0 by 0.5 cm., was grayish white and rubbery firm. A depressed area of scarring,

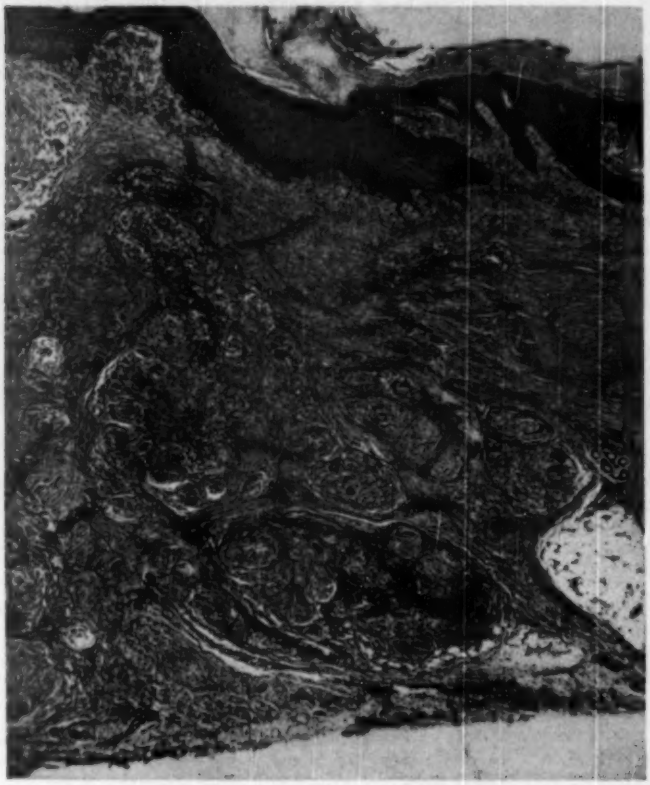

Fig. 1.—Low power view of the palmar scar, showing the discrete and confluent tuberculoid beryllium granulomas in the deep cutis and in the subcutaneous fat. Note the marked resemblance to the Darier-Roussy type of Boeck's sarcoid. × 40.

3 mm. in diameter, was present in the center of the skin surface. The tissue was fixed in formaldehyde solution U. S. P. diluted 1:10, sectioned at 6 microns, and stained with hematoxylin and eosin, Masson's trichrome stain, Foot's reticulum stain, the Verhoeff-Van Gieson elastic tissue stain and acid-fast stains for tubercle bacilli.

Histologically, the epidermis revealed hyperkeratosis, acanthosis, some elongation of the rete pegs and slight spongiosis, mainly in the stratum spinosum. The cutis was composed of dense parallel bundles of collagenous fibers, and in the papillary and reticular layers there were focal perivascular collections of lymphocytes and macrophages. In the remainder of the cutis, especially in the lower half, and extending down into the subcutaneous fat, there were numerous discrete and confluent tuberculoid granulomatous nodules embedded in a dense connective tissue stroma. The granulomatous process consisted of typical epithelioid cells, lymphocytes, macrophages and large, multinucleated giant cells of both Langhans and foreign body types. Many of the granulomas were well encapsulated by concentrically arranged bundles of collagenous fibers, and related to many of them were collections of lymphocytes. Connective tissue replacement was conspicuous, and all degrees, from early fibroblastic cell proliferation to almost complete fibrosis, were seen. A very few of the lesions showed small central foci of granular debris, but this was absent in most instances. No foreign body material was present, nor were any inclusion bodies noted in the giant cells. No doubly refractile matter was demonstrated. The acid-fast stains were negative. The Verhoeff–Van Gieson stain revealed loss of elastic fibers in the superficial areas of scarring, but they were seen about some of the granulomatous nodules. Foot's reticulum stain showed this substance to be present within some, but not all, of the nodules.[4]

COMMENT

The marked similarity in histologic appearance of beryllium granuloma and the Darier-Roussy type of Boeck's sarcoid is well shown in figures 1 and 2. In the absence of an adequate history it is doubtful whether the two can be differentiated on histologic grounds alone. In a study of beryllium pneumonitis, Dutra [5] considered certain features by means of which, in his opinion, differentiation was possible. These included the position of the giant cells, the number of nuclei in the giant cells, the presence or the absence of central necrosis and the relative numbers of epithelioid cells (see table).

An attempt to apply these criteria to the case herein presented emphasized not only the difficulty of assigning any diagnostic specificity to the histologic picture of this lesion but, even more, the morphologic similarity of the granulomatous reactions produced by apparently unrelated agents of disease. For instance, epithelioid cells were by no means rare; the giant cells were situated as often centrally as peripherally; the number of nuclei in the giant cells varied from 8 to 46, but half of such cells possessed 25 to 30 nuclei; fibrinoid or granular necrotic debris was rarely seen in the center of the granulomas.

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4. Spectographic analysis of a fragment of the original material was done by Mr. Harold P. Stephenson and Dr. H. Sponer, of the department of physics of Duke University. The method used was sufficiently sensitive to detect 2 parts of beryllium in 1,000,000 parts of solute; yet beryllium was not demonstrable in the present instance. Nevertheless, the history, the clinical course and the histologic appearance of the lesion are so characteristic of the subcutaneous beryllium granuloma that one cannot escape the conclusion that this indeed is an example of such a lesion.

5. Dutra, F. R.: Am. J. Path. **24**:1137, 1948.

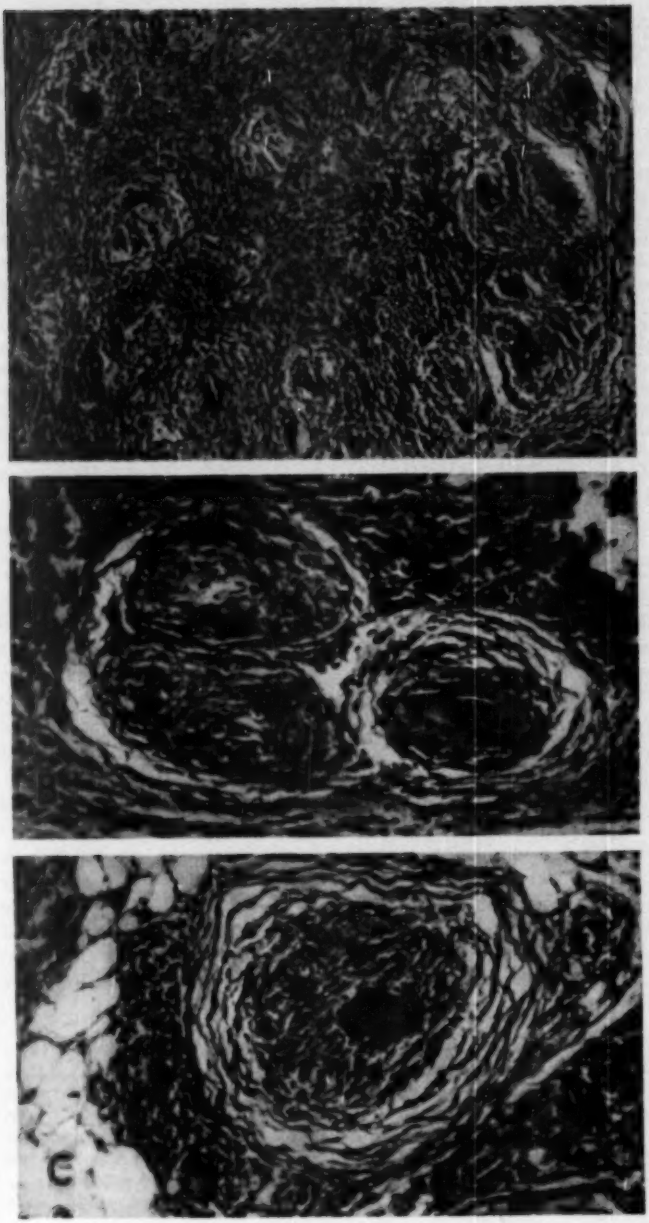

Figure 2.

(See legends on opposite page)

From a consideration of the foregoing facts it appears that although certain minor differences in the appearance of the lesions of Boeck's sarcoid of the Darier-Roussy type and beryllium granuloma may be

Features Described by Dutra[5] as Significant in the Differentiation Between Boeck's Sarcoid and Beryllium Granuloma

Sarcoid	Beryllium Granuloma
1. Compact mass of epithelioid cells	Typical epithelioid cells rare
2. Giant cells centrally located	Central giant cells rare
3. Giant cells large, with 25-30 peripheral nuclei	Giant cells vary, with 4-30 + nuclei
4. Loose perinodular connective tissue with numbers of lymphocytes	Granulomas infiltrated with moderate numbers of lymphocytes
5. No central necrosis	Most granulomas have fibrinoid material or granular necrotic eosinophilic debris centrally

present, yet the over-all pictures are so similar that differentiation on morphologic grounds alone seems highly unlikely.

SUMMARY

A case of subcutaneous beryllium granuloma is presented. The typical history of skin lacerated by a fluorescent light tube is recorded again as in 5 previously published cases. The remarkable similarity in histologic appearance of the beryllium granuloma and Boeck's sarcoid is emphasized.

EXPLANATION OF FIGURE 2

Fig. 2.—*A*, medium power view of the palmar scar. The giant cells, of both Langhans and foreign-body types, are situated centrally as well as peripherally. This view illustrates the fibrosis and lymphocytic infiltration, and the diffuse nature of the process. × 132.

B, high power view of three confluent lesions. Each is composed of giant cells, epithelioid cells, macrophages and a few lymphocytes, and is surrounded by concentric layers of collagenous fibers. Note the adjacent collection of lymphocytes. × 265.

C, a discrete tuberculoid granuloma with a centrally placed Langhans giant cell, macrophages, epithelioid cells and a few lymphocytes. The lesion is well encapsulated, is related at one point to a collection of lymphoid cells, and is situated in the subcutaneous fat. × 265.

EXPERIMENTAL ENDOCARDITIS WITH FIBRINOID DEGENERATION IN THE HEART VALVES OF RATS

Special Reference to the Fibrinoid

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FIBRINOID degeneration or "fibrinoid" has been found in various conditions [1]—in acute rheumatic inflammations (valves, Aschoff nodules, subcutaneous nodules, arthritis and pneumonitis), in acute and subacute bacterial endocarditis (heart valves), in rheumatoid arthritis with the associated subcutaneous nodules, in bursitis (the walls of the inflamed bursas), in periarteritis nodosa and temporal arteritis (the walls of the arteries), in peptic ulceration (the wall of the ulcer), in anaphylactic shock (the lesions of the arteries and the lungs) and in acute disseminated lupus erythematosus.

This fibrinoid degeneration or necrosis has been interpreted in various ways. Klinge [2] called it fibrinoid because it resembled fibrin but failed to stain in all respects like fibrin. Gross [3] called it degenerating collagen. Altshuler and Angevine [1a] have recently made chemical studies of this substance and have concluded that the common feature of fibrinoid formations is a precipitation of the acid mucopolysaccharide of the ground substance of connective tissue. It has come to be believed generally that fibrinoid degeneration does not represent any one etiologic entity.

The experiments reported in this paper were undertaken because of an interest in the cellular and fibrinoid reaction noted in the human heart valve in acute rheumatic fever and because in previous experiments [4] in which an attempt was made to produce endocarditis in rats the material in question was commonly seen in the vegetations. An effort was also made to see whether the incidence of the development of

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From the Department of Pathology, University of Minnesota.

This study was aided by a grant from the United States Public Health Service.

1. (*a*) Altshuler, C. H., and Angevine, M. A.: Am. J. Path. **25**:1061, 1949. (*b*) More, R. H., and McLean, C. R.: Am. J. Path. **25**:413, 1949.
2. Klinge, F.: Ergebn d. allg. Path. u. path. Anat. **27**:1, 1933.
3. Gross, L., and Ehrlich, J. C.: Am. J. Path. **10**:489, 1934.
4. Clawson, B. J.: Arch. Path. **40**:153, 1945.

endocarditis and the degree of fibrinoid bore any significant relation to any possible immune condition existing in the animal at the time of the blood stream inoculation.

MATERIALS AND METHODS

The rat was the animal used because in previous experiments it had been found possible to produce frequently an endocarditis very similar in structure to the endocarditis found in human rheumatic valvulitis, which is characterized by having an abundance of fibrinoid. Another reason for using the rat was that in this animal it has been found almost impossible to demonstrate by the intracutaneous skin test hypersensitiveness of either the anaphylactic or the tuberculin type.[5] It was thought that this fact might have some bearing in interpreting the effect of hypersensitiveness on the genesis of rheumatic and bacterial endocarditis.

Two types of animals were used in the experiments: normal animals and immune animals. Two methods of immunization were employed. The first consisted in several intraperitoneal injections of heat-killed streptococci. It was found in other experiments, in rabbits, that this method gave high titers of agglutinins but no bacterial hypersensitiveness. The second method was that used in previous experiments to develop bacterial or tuberculin type hypersensitiveness (delayed hypersensitiveness) in rabbits. This method was that recommended by Swift[6] and later used by me[7] with rabbits. It consisted in subcutaneous injections of 2 cc. of melted agar cooled to about 50 C. and heavily seeded with streptococci. In the previous experiments with rabbits treated by this method, after a period of about twelve days, when an intracutaneous injection of a small amount of heat-killed streptococci was given, a strong cutaneous reaction developed in most cases in twelve to twenty-four hours. In none of the rats tested was a positive skin test obtained. These results in rats were similar in respect to the bacterial type of hypersensitiveness, in general, to the findings of Hehre and Freund[5] in their experiments with the tuberculin type hypersensitiveness in rats, although they did obtain some evidence of hypersensitiveness when tuberculin was injected intraperitoneally. This seemed to indicate the possibility of some hypersensitiveness in rats even with a negative reaction to the skin test.

Agglutinins giving agglutination in serum dilutions of 1:6,400 to 1:25,000 developed in the serums of the rats receiving intraperitoneal injections and also in the rats which had received subcutaneous injections of agar and streptococci.

The organisms used in the attempt to produce humoral antibody immunity and bacterial hypersensitiveness, as well as the organisms employed in the later intracardiac injections, were a strain of alpha streptococci (Streptococcus viridans) isolated from the blood of a patient having acute rheumatic fever and two strains of beta hemolytic streptococci (Str. pyogenes) (Lancefield group A). The results obtained were approximately the same with the three strains. The number of intracardiac injections was from one to three in most cases. All of the 881 animals used in the experiments received these intracardiac injections.

The stain routinely used for the study of the cellular reaction and for determining the presence of fibrinoid was hematoxylin and eosin, but the Schiff reaction

5. Hehre, E., and Freund, J.: Arch. Path. **27**:289, 1939.

6. Derick, C. L.; Hitchcock, C. H., and Swift, H. F.: J. Exper. Med. **52**:1, 1930.

7. Clawson, B. J.: J. Infect. Dis. **53**:157, 1933.

(periodic acid in combination with the Schiff leukofuchsin reagent)[8] was applied in many cases in identifying the fibrinoid as similar to that seen in the vegetations in acute rheumatic fever.

THE RESULTS OF THE EXPERIMENTS

The results observed in the 881 rats following the intracardiac injections of streptococci are noted in the table. Two anatomic types of endocarditis were produced. The first is called the rheumatic-like

Fig. 1.—Human acute rheumatic valvulitis with fibrinoid which has broken through the endocardium.

Incidence of Rheumatic-like and Bacterial Endocarditis, With and Without Fibrinoid Degeneration, Following Intracardiac Injections (0.5 cc. Each) of a Broth Culture of Streptococci in Normal and Immunized Animals (881 Cases)

	Number Presenting Endocarditis of the Given Type								
	Rheumatic-Like				Bacterial				
	Cellular with Fibrinoid		Cellular Only		Cellular with Fibrinoid		Cellular Only	Total	
Animals	No.	%	No.	%	No.	%		No.	%
Normal (803)	80	13.2	34	5.6	75	12.4	0	189	31.3
Immunized (278)	21	7.5	4	1.4	16	5.7	0	41	14.7
Total (881)	101	11.4	38	4.3	91	10.2	0	230	26.1

8. McManus, J. F. A.: Am. J. Path. **24**:643, 1948.

endocarditis because of its close anatomic resemblance to the valvulitis found in cases of acute rheumatic fever (fig. 1). Within, and often extending outward and breaking through, the endocardium there was a definite fibrinoid change which by microchemical methods was found to be a saccharide similar to that found in the human acute rheumatic valvulitis. Surrounding the fibrinoid, a cellular reaction was noted. The cells were primarily large, somewhat irregular cells, mostly mononuclear, but multinucleated cells not infrequently were present. The

Fig. 2.—Rheumatic-like valvulitis of the rat showing fibrinoid in the center.

cells, to a less extent than in human rheumatic valvulitis, had a tendency to be arranged in a palisade formation. As in some cases of early rheumatic valvulitis, a cellular reaction was noted without any fibrinoid. Polymorphonuclear leukocytes were often seen but, as a rule, in small numbers. A small platelet thrombus extending outward from the surface of the valve, free from any bacteria, was often present.

The bacterial or second type of endocarditis produced a structure similar to that found in human cases. A large platelet thrombus containing many streptococci in colonies and fibrinoid degeneration occurring within the valves were noted in all.

Of the 603 normal animals which received the intracardiac injections, rheumatic-like endocarditis was found in 114 (18.9 per cent) and bacterial endocarditis in 75 (12.4 per cent). Endocarditis of one or the other type was present in 189 (31.3 per cent). These normal animals had not had previously infected or injured valves. In many controls spontaneous endocarditis was never observed. In 34 (5.6 per cent) of the 114 cases of nonbacterial endocarditis only a cellular proliferation without any fibrinoid was noted in the different sections of the valves examined. It is even possible that in some of these 34 cases fibrinoid was also present. It was present in all the vegetations of the bacterial type.

In the 41 animals which had previously been given intraperitoneal injections of streptococci and in the 237 animals which had been given subcutaneous injections of agar and streptococci, rheumatic-like endocarditis followed the intracardiac injections in 25 (8.9 per cent) and bacterial endocarditis in 16 (5.7 per cent). Endocarditis was seen in a total of 41 (14.7 per cent) of these animals. The cellular response and fibrinoid were observed in all but 4 of the animals showing rheumatic-like involvement and in all of those having the bacterial type. Of the 881 rats which had received the intracardiac injections, endocarditis with a high incidence of fibrinoid occurred in 230 (26.1 per cent).

It is to be noted that endocarditis occurred in 31.2 per cent of the normal animals and in 14.7 per cent of the immunized animals. The latter group, which had the smaller percentage of endocarditis, differed from the normal animals immunologically in having a striking increase in humoral antibodies as indicated by agglutinins. There was, however, no positive skin reaction to heated killed streptococci. A test for a possible visceral hypersensitiveness used by Hehre and Freund was not used.

COMMENT

The following points have been considered in the experiments: (1) the frequency and the structure of the experimentally produced vegetations on the valves, (2) the character of the animals used in the experiments, (3) the possibility that an immune state of the animals is related to the degree of involvement of the various structures, cellular and fibrinoid, in the experimental lesions and (4) the question whether in order for a vegetation to develop it is necessary that a valve be previously injured or infected.

Vegetations may commonly be produced after the intracardiac injection of streptococci. Many of these lesions have cellular and fibrinoid reactions which are anatomically identical with what is found in the valves in human acute rheumatic and bacterial endocarditis.

The rat has proved to be a valuable animal, for it has been possible to produce lesions more nearly simulating human lesions than those which have been produced by us in other animals, such as the rabbit and the monkey, and because it is an animal in which high concentrations of antibodies may be produced but hypersensitiveness either of the anaphylactic or the bacterial type cannot be demonstrated by a positive skin test.

The incidence of endocarditis, either rheumatic or bacterial, is less in animals which have high titers of antibodies, but the possible presence of either type of hypersensitiveness, immediate or delayed, cannot be demonstrated to have any significance in the development of the types of vegetations reported in the experiments.

Another conclusion to be drawn, especially with regard to rats, is, as was found by Dick and Schwartz [9] in dogs, that it is not necessary to have a previously injured or infected valve in order for endocarditis to develop. The endocarditis produced in these experiments developed on normal valves following one to three intracardiac injections of Str. viridans or Str. pyogenes. It was found impossible to produce lesions in 35 other rats by injecting into the left ventricular cavity several other substances, as horse serum, rabbit serum, egg albumin and Dick toxin. It appears evident that the lesions found after the intracardiac injection of streptococci were not due in any way to the possible mechanical injury of the needle going into the heart.

The two types of endocarditis, rheumatic and bacterial, are commonly found together on the same valve in cases of subacute bacterial endocarditis. In human cases it cannot be determined definitely whether the two types of vegetations are due to the same cause or whether two separate diseases are present on the valves at the same time. In the rat it is apparent that the two lesions anatomically similar to the human lesions are due to the same cause and may develop at approximately the same time.

It is obvious, however, that the rheumatic-like vegetations which have frequently been produced in these rats are not necessarily etiologically the same as the lesions found in human valvulitis, for it is now known that the cellular response and especially the fibrinoid similar to what is exhibited in these rat valves may result from various causes and in various conditions.

It can only be suggested that some evidence has developed from these experiments concerning the causation of rheumatic fever. An easy and efficient method has been demonstrated which may be used in the further study of proliferative inflammation of the heart valves with the associated fibrinoid degeneration.

9. Dick, G. F., and Schwartz, W. B.: Arch. Path. **42**:159, 1946.

SUMMARY

Endocarditis with fibrinoid degeneration can be produced experimentally in the rat in a relatively high percentage of cases by intracardiac injections of alpha or beta hemolytic streptococci (Str. viridans or Str. pyogenes).

Two anatomic types of vegetations, rheumatic-like and bacterial, were observed. The bacterial vegetations had colonies of bacteria in the platelet thrombus on the valve.

The presence of fibrinoid giving a positive Schiff reaction was noted in most of the cases of rheumatic-like and in all of the cases of bacterial endocarditis.

The development of either kind of endocarditis did not appear to depend on streptococcic hypersensitiveness as far as could be determined by an intracutaneous skin test.

The incidence of endocarditis was less in animals with a high concentration of streptococcic antibodies in their serums than in normal animals.

The rat, in which no type of hypersensitiveness can be demonstrated by means of an intracutaneous skin test, has been shown in our experience to be the best animal in which to produce rather commonly an endocarditis resembling human rheumatic endocarditis.

GLOMERULAR LESIONS IN THE DIABETIC RAT

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KIMMESTIEL and Wilson[1] in 1936 described sclerosing lesions of the renal glomerulus in diabetes mellitus in man. Many works have confirmed this finding, but there have been differences of opinion as to the specificity of the lesions, especially when a comparison is made with the lesions in hypertensive vascular diseases, nephrosclerosis and chronic glomerulonephritis.[2] Similar lesions have been observed in dogs with pituitary diabetes which were kept alive for a number of years.[3]

This paper deals with histologic observations made on the glomerular elements of the kidneys of rats after partial pancreatectomy. One[4] of us has shown that in the male white rat in which 95 per cent of the pancreas has been removed diabetes follows a peculiar course, which has been divided into three successive stages: The first—prediabetes—extends from the time of operation to the beginning of alimentary glycosuria, generally about one month. The animal is free from diabetic symptoms and appears normal. The blood sugar is normal after seven hours of fasting. The second stage—incipient diabetes—starts with the appearance of alimentary glycosuria and ends with the development of fasting hyperglycemia. It lasts two to three months and is characterized by successive appearance of all the diabetic symptoms. The third stage—confirmed diabetes—commences when there is fasting hyperglycemia after seven hours of fasting and ends with the death of the animal several months or as much as one year later. In this

From the Instituto de Biologia y Medicina Experimental, Costa Rica 4185.

1. Kimmestiel, P., and Wilson, C.: Am. J. Path. **12**:83, 1936.

2. (*a*) Allen, A. C.: Arch. Path. **32**:33, 1941. (*b*) Horn, R., and Smetana, H.: Am. J. Path. **18**:93, 1942. (*c*) Bell, E. T.: Ibid. **18**:744, 1942. (*d*) Laipply, T.; Eitzen, O., and Dutra, F. R.: Arch. Int. Med. **74**:354, 1948. (*e*) Spuehler, O.: J. Clin. Endocrinol. **5**:325, 1945. (*f*) Bogliolo, L.: Arch. Soc. Argent. de anat. norm. y pat. **7**:35, 1945.

3. Lukens, F. D. W., and Dohan, F. C.: Arch. Path. **41**:19, 1946.

4. Foglia, V. G.: Rev. Soc. argent. de biol. **20**:21, 1944.

stage the rats show frank polyuria, increased weight of the kidneys [5] and glomerular sclerosis.[6] Only one third of the females subjected to this operation showed diabetes, and in these the first and second stages were much longer than in the control males.

MATERIAL AND METHODS

All the experiments were done on white rats from the stock colony kept at this institute for the past thirty years. They were maintained on the ordinary diet. Ninety-eight animals were used, 84 males and 14 females. Pancreatectomy was carried out by one of us (V. G. F.) when they were 3 months old. In some rats approximately 95 per cent of the pancreas was removed, and in a small group, about 98 to 100 per cent. The technic employed has been described elsewhere.[4]

The male rats were divided into four groups, as follows: (1) normal controls—20 animals; (2) pancreatectomized (95 per cent) rats with normal blood sugar (prediabetes)—10 animals; (3) pancreatectomized (95 per cent) rats in a more advanced stage with hyperglycemia (confirmed diabetes)—49 animals; (4) pancreatectomized rats in which more than 95 per cent of the pancreas was removed, causing the immediate appearance of hyperglycemia—5 animals.

The female rats were divided into two groups: (5) pancreatectomized (95 per cent) with normal blood sugar (prediabetes)—6 animals; (6) pancreatectomized (95 per cent) with hyperglycemia (confirmed diabetes)—8 animals.

At monthly intervals the animals were fasted for seven hours, after which blood was taken from the tip of the tail and its sugar content determined by the method of Hagedorn and Jensen.

The animals were killed with illuminating gas, and a kidney of each one was fixed in Bouin's fluid, Helly's fluid and 4 per cent formaldehyde solution. The following stains were used: hematoxylin-eosin; Mallory-Heidenhain azocarmine, Van Gieson; Hortega's silver impregnation for reticular fibers; congo red and methyl violet for amyloid; Pinkus technic for elastic fibers; sudan III and scarlet red for fat; the Altmann-Gersh fixation (freezing, drying) and iodine reaction for glycogen (Mancini [7]), and the periodic leukofuchsin technic (McManus [8]; Hotchkiss [9]), thionine and toluidine blue for mucoproteins. Some slices were exposed during twenty-four hours to the action of a 0.1 per cent aqueous solution of hyaluronidase and after that were stained with mucin technics.

RESULTS

The results of the histologic investigations of the kidneys of these animals are considered in the following order: (1) the normal glomeruli, (2) the glomeruli of the diabetic rat and (3) the distribution of the glomerular lesions in the different groups of male and female rats at various intervals after operation.

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5. Foglia, V. G.: Rev. Soc. argent. de biol. **21**:45, 1945.

6. Foglia, V. G.; Mancini, R. E., and Cardeza, A. F.: Rev. Soc. argent. de biol. **24**:114, 1948.

7. Mancini, R. E.: (*a*) Medicina **4**:7, 1947; (*b*) Arch. Soc. argent. de anat. norm. y pat. **9**:188, 1947; (*c*) Anat. Rec. **101**:149, 1948; (*d*) Rev. Soc. argent. de biol. **25**:190, 1949.

8. McManus, J. F. A.: Nature **158**:202, 1946.

9. Hotchkiss, R.: Arch. Biochem. **16**:131, 1948.

Normal Glomeruli of the Control Rats.—The wall of the afferent arteriole, the visceral membrane forming the walls of the capillaries of the glomerular tuft, and the parietal basal membrane which derives from Bowman's capsule were delicate (fig. 1 *A* and *B*). They stained deep blue with Mallory-Heidenhain azocarmine (fig. 1 *B*), pink with Van Gieson's stain and purplish red with the periodic leukofuchsin technic (fig. 1 *C*) and did not assume a metachromatic color with thionine or toluidine blue. The silver impregnation brought out a fine reticulum at the hilus with occasional branching at the periphery of the glomerular tufts (fig. 1 *D*). Neither glycogen nor fat was observed. In the visceral basal membrane deeply stained folds were noticed, forming the so-called "mesangium," [10] and there were some distally extending fine prolongations of the hilar reticulum and infrequent cells (fig. 1 *D*) ("axial space" [11]).

Changes in the Glomeruli of Diabetic Rats.—Glomerular lesions usually were observed in the pancreatectomized rats. They passed through two successive stages, presclerotic and sclerotic.

Presclerotic Change.—As a rule, all the glomeruli increased in size. The capillary tuft appeared unfolded, and the capillaries were widened and usually engorged with red cells. Bowman's space was narrowed, and in places the capillary tuft sometimes came in contact with the parietal membrane. The intraglomerular portion of the afferent artery, the walls of the capillaries and particularly the "mesangium" became thicker and hyaline. The three took a blue-reddish or deep blue color with Mallory-Heidenhain azocarmine, light brown with Van Gieson's stain and deep red with the periodic leukofuchsin technic. Also, the metachromatic staining was negative.

Occasionally some of the capillaries adhered to one another, and the same was true of the "mesangiums."

Both the parietal basal membrane of Bowman's space and the proximal convoluted tubules were usually thickened and had identical staining properties. Conversely, the preglomerular afferent artery did not show changes with the same technics. Silver impregnation disclosed the same fine reticulum in the hilus and the "mesangium" as in the controls. The cells of the visceral basal membrane were hypertrophic, and their cytoplasm contained granules of glycogen but no fat.

As time went on all the lesions became gradually more pronounced, some of the glomeruli having both the visceral and the parietal membrane partially fused together. Simultaneously there was an increase in reticular fibers in the hilus and the "mesangium."

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10. Zimmerman, K. W.: Ztschr. f. mikr.-anat. Forsch. **32**:176, 1933. Bensley, R. R., and Bensley, R. D.: Anat. Rec. **47**:147, 1930. McGregor, L.: Am. J. Path. **5**:545, 1929.

11. McManus, J. F. A.: Am. J. Path. **19**:642, 1948.

Sclerotic Change.—The glomerular tuft appeared shrunken. This was due to the retraction of the capillaries, which were collapsed and ischemic, and sometimes distinctly atrophic (fig. 2*A*). The intraglomerular portion of the afferent artery, the walls of the capillaries and particularly the "mesangium" were thicker and less hyaline than previously. Silver staining revealed that this thickening was due to

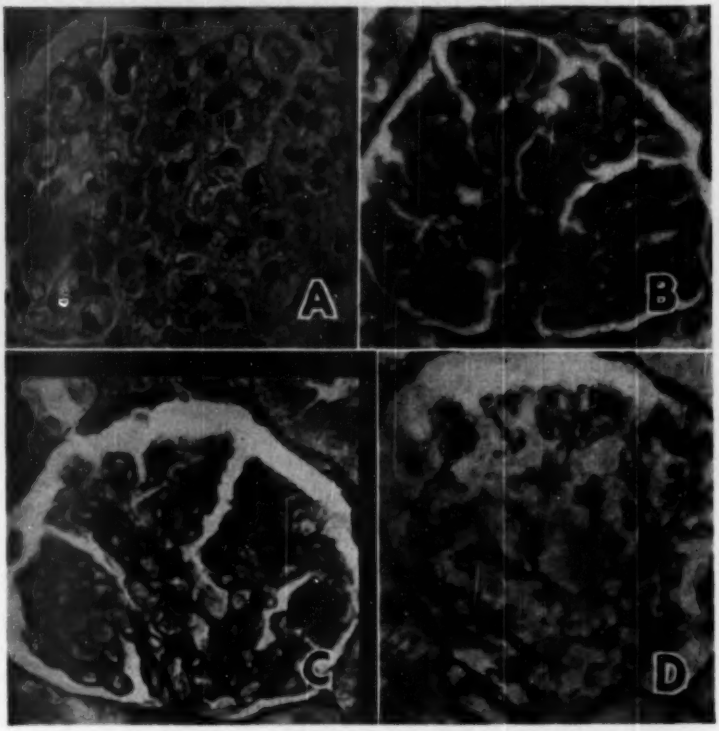

Fig. 1.—Glomeruli of normal control rats. Observe the fine visceral basal membrane forming the "mesangium" and the capillary walls. The basal membrane is clearly seen. The recticular fibers which branch out from the hilus to the "mesangium" are very scanty. *A*, hematoxylin-eosin stain. *B*, Mallory-Heidenhain azocarmine stain. *C*, periodic leukofuchsin technic. *D*, Rio Hortega's silver impregnation for reticulum. Magnifications, × 540.

a striking increase in reticular fibers extending from the hilus through the "mesangium" into the glomerulus, and coming to surround also the capillaries (fig. 2*D*). These thickened structures stained deep blue

with Mallory-Heidentain azocarmine (fig. 2*B*) and reddish with Van Gieson's stain. With the periodic leukofuchsin technic the basal membrane, the "mesangium" and the capillaries were distinctly seen but were then shrunken and atrophic (fig. 2*C*). The metachromatic stain was still negative. In no case did hyaluronidase digestion modify the results of the aforementioned technics for mucin. The cells of the

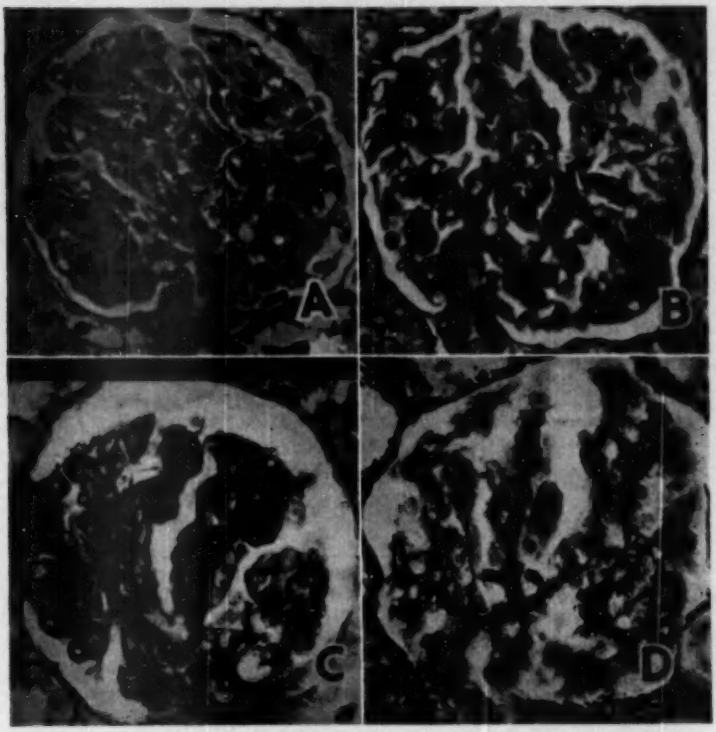

Fig. 2.—Glomeruli of rats twelve months after pancreatectomy. Note the fibrillar thickening of the wall of the intraglomerular afferent arteriole, of the "mesangium" and of the walls of capillaries. The basal membrane is folded and atrophic. *A*, hematoxylin-eosin stain. *B*, Mallory-Heidenhain azocarmine stain. *C*, periodic leukofuchsin technic. *D*, Rio Hortega's silver impregnation for reticulum. Magnifications, × 540.

basal visceral membrane were atrophic, and fibroblast-like cells were seen among the fibers of the "mesangium." Interstitial sclerosis was noted between the convoluted tubules, many of which appeared atrophic.

In no instance was any alteration noted in the wall of the extraglomerular portion of the afferent artery or in the walls of the other arteries of the interstitial tissue of the kidney.

Distribution of the Glomerular Lesions in the Different Groups of Animals (table).—(*a*) Pancreatectomized (95 per cent) Male Rats (groups 2 and 3): During the first two months, when the blood sugar was still at the normal level, the glomeruli revealed no difference in structure when compared with those of the controls.

From the second month on, as the blood sugar increased (120 to 350 mg. per hundred cubic centimeters), hyaline thickenings of the

Incidence of Glomerular Lesions in the Kidney of the Rat After Subtotal Pancreatectomy

	Group	Rats	Glycemia, Mg. per 100 Cc.	Months After Pancreatectomy	Rats Without Lesions	Rats with Lesions		
						Presclerosis	Sclerosis	%
Males								
1	Normal controls	20	80-130	...	20	..	..	..
2	Pancreatectomized (95%) with normal blood sugar (first stage)	10	80-130	1-2	10	..	..	..
3	Pancreatectomized (90%) with hyperglycemia (third stage)	49	130-350	2-12	6	26	17	88
4	Pancreatectomized (greater than 90%) with hyperglycemia (acute diabetes)	5	120-250	1-2	5	..	..	..
Females								
5	Pancreatectomized (90%) with normal blood sugar (first stage)	6	80-130	1-11	6	..	..	..
6	Pancreatectomized (95%) (castrated) with hyperglycemia (third stage)	8	130-410	0-6	1	6	1	88

glomeruli began to appear (presclerosis) and became more pronounced with time until sclerosis set in. In general, four months after pancreatectomy the hyaline thickenings were easily seen. The table shows that lesions of these two types were present in 88 per cent of the rats. In this group 53 per cent of the lesions were presclerotic and 35 per cent sclerotic. In the remaining 12 per cent, despite the time which had elapsed since operation, there were no lesions, but the blood sugar rose no higher than 200 mg. per hundred cubic centimeters. In those pancreatectomized animals in which diabetes did not develop, glomerular alterations were not demonstrable.

(*b*) Male Rats with Pancreatectomy of More Than 95 per Cent (group 4): In spite of the high blood sugar (120 to 250 mg. per hundred cubic centimeters) there were no visible lesions during the first two months after the operation.

(*c*) Female Rats with Pancreatectomy of 95 per Cent (group 5): During the eleven months following the pancreatectomies the blood sugar levels were inconsistent and no higher than 140 mg. per hundred cubic centimeters. The glomeruli remained unchanged.

(*d*) Castrated Female Rats with Pancreatectomies of 95 per Cent (group 6): The blood sugar was high (120 to 410 mg. per hundred cubic centimeters), and after six months lesions appeared. The incidence of presclerotic lesions was higher than that of sclerotic ones.

COMMENT

The results show that the glomerular lesions were progressive, there being at first a hyaline thickening of the "mesangium," of the walls of the capillaries and of the intraglomerular afferent arteriole. Later the hyaline change was replaced by diffuse sclerosis. The lesions appeared only in those rats which had undergone partial pancreatectomy and were diabetic. The 20 normal controls (group 1) did not show any alterations.

The lesions appeared some time after the operation, usually after the second month, in 88 per cent of the animals with high blood sugar (groups 3 and 6). A close correlation of the level of the blood sugar and the severity of the lesions could not be established, although the lesions were usually more severe the higher the glycemia and the greater the lapse of time after the operation.

In those pancreatectomized animals in which diabetes did not develop no renal lesion was found (groups 2 and 5). In the animals in which more than 95 per cent of the pancreas was removed (group 4) and in which there was immediate diabetes with high blood sugar, no glomerular lesions were found when the animals were killed two months later. Therefore, it appeared that the lesions developed slowly, probably through the action of prolonged hyperglycemia.

In female rats with pancreatectomy of 95 per cent (group 5) diabetes did not develop during their eleven months' survival, nor did glomerular lesions occur. In castrated female rats in which 95 per cent of the pancreatic tissue was removed, diabetes and glomerular lesions occurred before the sixth month. These results are in accord with the recognized fact that pancreatectomy fails to produce diabetes in female rats as often as it does in males.[12] This is probably due to the protective action of the ovaries, since their removal leads to the appearance of diabetes.[13] The question whether there are some other metabolic factors, besides the hyperglycemia, which can influence and produce the glomerular lesions in the diabetic rat has not as yet been studied.

12. Foglia, V. G.: Rev. Soc. argent. de biol. **21**:360, 1945.

13. (*a*) Foglia, V. G.; Schuster, N., and Rodriguez, R. R.: Endocrinology **41**:428, 1947. (*b*) McManus, J. F. A.: Am. J. Path. **19**:1259, 1948.

Studies in progress indicate that there are no noticeable alterations in the preglomerular afferent artery, the vessels of the interstitial tissue of the kidney or the vessels of other organs. Glycogen appears in the distal convoluted tubule and Henle's loop only during the period of "confirmed diabetes." Small amounts of fat appear in the proximal convoluted tubule at the end of the same period. There is an increase in the glycogen of the leukocytes of the blood at the confirmed period of the diabetes, and a disappearance of the small content of glycogen in the fat cells of the adipose tissue of the groin, the back, the neck and the perirenal fat.[7d]

Histologically the glomerular lesions are interpreted as being a simultaneous hyaline thickening in the wall of the intraglomerular afferent arteriole, the "mesangium" and the capillaries. The hyaline thickening of the presclerotic lesion is interpreted as an alteration of the intraglomerular connective system, that is, an alteration of the reticular fibers of the wall of the intraglomerular afferent arteriole, the "mesangium" and the capillary basal membrane. Histochemically these hyaline thickenings are glycoproteins, for they stain deep red with the periodic leukofuchsin technic; nevertheless, it does not seem to be hyaluronic acid either. The periodic leukofuchsin reaction is also obtained in the normal glomerulus but in less amount.[12b] On the other hand, the staining for amyloid revealed none.

The sclerosis is due to an increase of the reticular fibers. These fibers are present in the hilus of the glomerulus and, more abundantly, in the "mesangium" and surrounding the walls of the capillaries.

The sclerotic lesion has the following characteristics: (*a*) It is a neoformation of reticular fibers and is accompanied by a proliferation of cells with a fibroblastic appearance; (*b*) it takes place in the same regions as the previous hyaline thickening; (*c*) it causes partial atrophy of the glomerulus. We believe that the hyaline thickening is the first step in the formation of reticular fibers in the glomerular lesion and that the fibroblast-like cells observed in the "mesangium" take part in this process. The change resembles the fibrillogenesis in mesenchymal tissues, being preceded by a stage in which an amorphous substance appears that stains intensely with the technics for glycoproteins.[14] The sclerotic lesion of the glomerulus of the rat appears to be similar to the alteration of the glomerulus in the patient with diabetes (especially the patient with the mild, so-called diffuse lesion [3d]) so far as distribution and tinctorial affinities are concerned. The lesion differs from that in the glomerulus of the rat with experimental nephrogenic hypertension,[15]

14. Bensley, R. R.: Anat. Rec. **60**:93, 1934. Meyer, K.: Physiol. Rev. **27**:335, 1947.

15. Gaudino, M.: Hipertensión nefrógena experimental, tesis, Buenos Aires, 1944.

first, in the greater severity of the latter; second, in the morphogenesis; third, in the site of origin, which is in the preglomerular afferent artery, and, fourth, in the pronounced fibrosis of the interstitial arterioles of the kidney.

SUMMARY AND CONCLUSIONS

Diabetes induced by 95 per cent extirpation of the pancreas in male and female rats causes lesions in the glomeruli in 88 per cent of the animals.

These lesions appear only in cases of definite and long-standing hyperglycemia (two to twelve months).

In general the glomerular lesions are more severe with higher glycemia and longer lapse of time after the operation.

In rats which were operated on but in which diabetes did not develop, glomerular lesions did not develop.

Animals with acute diabetes following extensive pancreatectomy did not show alterations during the ensuing two months.

Lesions began as hyaline thickenings of the intraglomerular afferent arteriole, of the "mesangium" and of the walls of capillaries, and later progressed to sclerosis. The hyaline substance in these lesions was stained with the technic used for mucopolysaccharides and did not take the stain for amyloid. The sclerosis was due to an increase in reticular fibers.

BENIGN NEOPLASMS OF THE EXTRAHEPATIC BILIARY DUCTS

Review of the Literature and Report of a Case of Fibroma

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BENIGN neoplasms of the extrahepatic biliary ducts are great rarities and, consequently, have received little attention in current literature (surgical, clinical and neoplastic treatises). Yet, by reason of their strategic situation, these seemingly innocuous growths may become dangerous lesions, for their mechanical encroachment on the lumen of the biliary passage when neglected may produce the same alarming effects and irreparable damages as their cancerous counterparts. Under early and adequate management, however, their characteristic benignity, their tendency to remain dormant for long periods, and their ready amenability to surgical removal render a favorable prognosis. For these reasons and because of the dearth of current reports, it is proposed (1) to describe a recent case of fibroma of the common hepatic bile duct, (2) to review the literature regarding histologically proved cases and (3) to discuss briefly the pathologic and clinical aspects.

The rare occurrence of these neoplasms is attested by the fact that in 1929 Rolleston and McNee [1] succeeded in collecting only 10 cases from the world literature while, at the same time, acceding 112 cases of carcinoma of the bile ducts, exclusive of the ampulla of Vater. Furthermore, in the twenty year period ending January 1930 Marshall [2] was able to accumulate only 4 cases of benign ductal new growths at the Mayo Clinic, compared with 49 cases of cancer, in approximately 20,000 surgical operations on the biliary tract. In 1933 Christopher [3] collected 41 cases from the world literature, to which he added a case of adenoma of the ampulla of Vater.

In the past sixteen years 7 additional cases have been reported. Leriche,[4] in 1934, reported a massive papillomatous tumor of the choledochus, weighing 750 Gm., in a child 4½ years old. McIntosh and

From the Department of Pathology, Jefferson Medical College.

1. Rolleston, H., and McNee, J. W.: Diseases of the Liver, Gall-Bladder, and Bile Ducts, ed. 3, London, The Macmillan Company, 1929, p. 738.

2. Marshall, J. M.: Surg., Gynec. & Obst. **54**:6, 1932.

3. Christopher, F.: Surg., Gynec. & Obst. **56**:202, 1933.

4. Leriche, R.: Lyon chir. **31**:598, 1934.

Gillies,[6] in 1940, reported a primary benign papilloma involving the common hepatic duct in a 50 year old woman. In 1947 de la Flor and B. Baraco [6] recorded a case of polyp of the common bile duct complicating lithiasis in a 38 year old woman. In 1940 Henry [7] reported a case of papilloma of the bile duct, the exact site of which was not specified. Siniscalco,[8] in the following year, presented a case of adenomatous polyp of the common bile duct in a 52 year old woman with progressive obstructive symptoms. Meade,[9] in 1945, described a case of papilloma of the common bile duct, but this was in association with a cancer of the common bile duct. Finally, Rogers,[10] in 1946, recorded a case of papillary cystadenoma of the common hepatic duct in a 41 year old multiparous woman.

REPORT OF CASE

A. D., a 45 year old white man, a meat salesman, entered the Jefferson Medical College Hospital with chief complaints of nausea, progressive jaundice, clay-colored stools, dark urine, anorexia and upper abdominal pain, which had been present for about five weeks. The patient's illness began five weeks prior to admission with the onset of weakness and nausea in association with a gradually increasing yellowish discoloration of the skin. A few days later he experienced a dull ache which extended along the right costal margin and a crampy pain in the epigastrium, which simulated gas pain. Nausea was quite intense but was not associated with vomiting. The patient consulted a physician and was sent to a hospital for study. He was discharged after a week, with the diagnosis of catarrhal jaundice. The pain, nausea and jaundice, however, did not subside, and, in addition, clay-colored stools and dark urine were noted. The disease became progressively worse and the patient was admitted to the Jefferson Medical College Hospital.

The patient had been a "fairly heavy drinker" for many years. There was no history of injections or transfusions having been given in the six months prior to admission.

The patient was well developed and fairly well nourished. There was moderate generalized icterus. The liver was tender and just palpable. There were no other significant physical findings. The temperature was 99.8 F.; the pulse rate was 70 and the respiratory rate 20.

On the first hospital day, biliary drainage (the tube being placed with the aid of the fluoroscope) was attempted, but only a small amount of light yellow-tinged fluid was obtained. Radiologic studies of the stomach and duodenum revealed no gross pathologic change. Laboratory studies disclosed 80 per cent retention of dye in the sulfobromophthalein excretion test; serum bilirubin 24.8 mg. per hundred cubic centimeters; urinary urobilinogen dilution, 1:20; van den Bergh test, direct positive for bilirubin; cephalin cholesterol flocculation, 2 plus; thymol turbidity, 8.4 units; plasma proteins, 6.57 Gm., with albumin 4.97 Gm. and globulin 1.6 Gm., per hundred cubic centimeters and a prothrombin time of 53 per cent.

5. McIntosh, A. M., and Gillies, A. D.: M. J. Australia **1**:268, 1940.
6. de la Flor, V. J., and Baraco, G. V.: Arch. peruanos pat. y clin. **1**:341, 1947.
7. Henry, C. K. P.: Canad. M. A. J. **42**:251, 1940.
8. Siniscalco, O.: Rev. méd.-quir. de pat. fem. **17**:260, 1941.
9. Meade, H. S.: Irish J. M. Sc., December, 1945, p. 710.
10. Rogers, K. E.: Canad. M. A. J. **55**:597, 1946.

During the next four days the patient improved considerably; his appetite returned, and the stools became light yellow to brown. The liver, however, remained tender and palpable. On the seventh day he suddenly became worse with return of tenderness in the right upper quadrant of the abdomen and clay-colored stools. Laboratory studies at this time revealed serum bilirubin 13.8 mg. per hundred cubic centimeters; urinary urobilinogen dilution, 1:50; retention of dye in the sulfobromophthalein test, 20 per cent; total plasma protein, 6.04 Gm. with albumin 4.14 Gm. and globulin 1.9 Gm. per hundred cubic centimeters; thymol

Fig.1.—Photograph of the exposed hepatic, cystic and common bile ducts. Note the fibroma in the wall of the common hepatic duct.

turbidity, 7.4 units, cephalin-cholesterol flocculation, 4 plus, and a prothrombin time of 33 per cent, which rapidly decreased to 25 per cent.

On the ninth hospital day, the stools became pigmented again, and the patient felt a little more comfortable. Subsequently, however, his condition progressively deteriorated, and he died in coma on the eighteenth day after admission.

Necropsy (ten hours after death).—The body was that of a well developed and well nourished, markedly jaundiced, white man. Approximately 2,500 cc. of dark yellow clear watery fluid was present in the abdominal cavity. The subcutaneous tissue and the abdominal viscera were stained deep yellow.

The liver weighed 1,100 Gm. and was small, soft and flabby. The capsule was smooth and shiny. The organ cut without difficulty and revealed an irregular exposed surface containing dark red central areas surrounded by light yellow-brown peripheral zones. The lobular markings were indistinct. The gallbladder was markedly distended and contained approximately 200 cc. of viscid, light green bile. Calculi were not present; the mucosa was smooth and stained yellow-green. The cystic duct was bound by dense fibrous tissue to the hepatic duct over a distance of 3 cm. proximal to the junction of the cystic and common hepatic ducts. The common hepatic duct and its left and right tributaries at the hilus of the liver were greatly distended and dilated. At a point 2.5 cm. proximal to the junction of the cystic and common hepatic ducts, a firm ovular nodule, measuring 1 by 0.8 by 0.8 cm., was found in the lateral wall of the common hepatic duct, causing com-

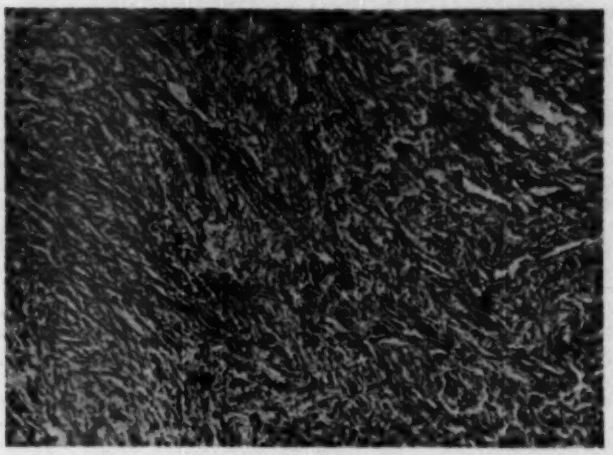

Fig. 2.—Section of fibroma of the common hepatic duct, showing the closely arranged fibrocytes composing the tumor. Hematoxylin and eosin; × 100.

pression and almost complete obliteration of the lumen (fig. 1). A fine probe was passed through this site with difficulty. It was first thought that this nodule represented a sentinel lymph node, but on section the mass was seen to be intramural in location and presented a compact, grayish yellow cut surface not unlike firm adipose tissue. No distinct capsule was discernible. The common bile duct was patent in its entirety and contained neither stones nor sand.

The kidneys were large and soft, and weighed 270 Gm. each. The capsules were stripped without difficulty, revealing a smooth, dark green-brown external surface. Cut surfaces bulged on cutting, disclosing barely discernible corticomedullary markings in a ratio of 1 to 3. The cortex was light greenish brown, while the medulla was of a darker greenish brown color with distinct longitudinal dark red streaks. Calices, pelves and ureters were stained yellow.

The stomach showed numerous submucosal petechiae. Clay-colored stool was present in the sigmoid colon and rectum. The other organs showed no significant gross pathologic changes.

Histopathologic Studies.—The tumor nodule from the common hepatic duct revealed a circumscribed mass of closely arranged, elongated, thin, spindle-shaped cells, suggesting fibrocytes (fig. 2). They were disposed in interlacing bundles and were of regular size, shape and staining. The cytoplasm was scanty, and the nuclei were darkly stained and fusiform. No mitotic figures or bizarre nuclear forms were evident. Scattered within the mass of fibrous tissue cells were several small vessels filled with erythrocytes. The mucosa of the duct overlying the nodule showed necrosis and atrophy from compression by the fibromatous tumor. The subserosa was similarly compressed. The muscular layer was not apparent, and a thin, compressed fibrous capsule surrounded the nodule. Lateral to the tumor, the subserosa contained a few focal collections of lymphocytes with occasional plasma cells.

Sections of the liver demonstrated the characteristic changes of biliary cirrhosis. There were diffuse necrosis of the liver parenchyma, marked fibrosis of the perilobular areas and conspicuous bile duct proliferation. Some of the bile ducts were distended and filled with inspissated masses of yellow-brown bile, and some of the periportal areas were infiltrated by lymphocytes.

The kidneys showed well preserved and normal glomeruli. Numerous yellow to brown-pigmented casts were present in the tubular lumens. The convoluted tubules, particularly the proximal portions, showed prominent degenerative changes of the epithelium.

Diagnosis.—Fibroma of the common hepatic bile duct; advanced biliary cirrhosis; bile nephrosis.

COMMENT

Although rare, benign neoplasms of the extrahepatic biliary ducts are exceedingly varied in types. New growths reported by different authors include papilloma and polyp,[11] adenoma,[12] fibroma,[13]

11. (*a*) Pozzi, G.: Gaz. med. ital. lomb. **2**:389, 1880; cited by Konjetzny, G. E.: Ergebn. d. allg. Path. u. path. Anat. **14**:714, 1910. (*b*) Toelg, J., and Neusser, E.: Ztschr. f. klin. Med. **7**:321, 1884. (*c*) Jourdan, M.: Bull. Soc. anat. de Paris **66**:323, 1891. (*d*) Chappet, V.: Lyon méd. **76**:145, 1894. (*e*) Rolleston, H. D.: Tr. Path. Soc. London **45**:83, 1894. (*f*) Monari, A.: Clin. med. ital. **37**:289, 1898. (*g*) McPhedran, A., in Sajouss C. E. de M.: Annual and Analytical Cyclopædia of Practical Medicine, Philadelphia, F. A. Davis Co., 1898-1901, vol. 4, p. 422. (*h*) Krause: Inaugural Dissert., Kiel, 1901; cited by Konjetzny.[11a] (*i*) Wilks and Moxon, cited by Rolleston and McNee.[1] (*j*) Eve, F.: Tr. Clin. Soc. London **39**:144, 1906. (*k*) Tedenat: Cong. franç. de chir. **5**:189, 1908; cited by Savy, P., Bonnet, P., and Martin, J. F.: Lyon chir. **9**:679, 1913. (*l*) Stein, J.: Prag. med. Wchnschr. **35**:383, 1910. (*m*) Pallasse: Soc. méd. d. hôp. de Lyon, cited by Savy and others.[11k] (*n*) Sommer, R.: Beitr. z. klin. Chir. **138**:357, 1926. (*o*) Archibald, E.: Ann. Surg. **92**:662, 1930. (*p*) Bazin, A. T.: Ibid. **92**:658, 1930. (*q*) Marshall, J. M.: Proc. Staff. Meet., Mayo Clin. **6**:191, 1931. (*r*) Goebel: Zentralbl. f. Chir. **52**:1654, 1925.

12. (*a*) Keen, W. W.: Boston M. & S. J. **126**:405, 1892. (*b*) Calzavara, C.: Arch. f. path. Anat. u. Physiol. **141**:221, 1895. (*c*) Volmer, A.: Arch. f. klin. Chir. **86**:160, 1908. (*d*) Menetrier, in Brouardel, P. C. H., and Gilbert, A.:

lipoma,[14] neuroma,[15] granuloma,[16] melanoma[17] and carcinoid.[18] In the present report an attempt was made to collect all the cases published in the literature. Accordingly, 54 cases were abstracted, including several omitted in earlier reviews. There were a few, however, the reports of which were not available, and others undoubtedly escaped notice, especially those that may have been reported in monographs, obscure journals or under misleading titles.

Including the case reported herewith, the 55 cases of benign tumors are disposed by type and frequency as follows: papilloma and polyp, 24; adenoma, 18; fibroma, 3; lipoma, 3; neuroma, 3; granuloma, 2; melanoma, 1; carcinoid 1.

Many among the reported cases lacked histologic confirmation, and a few, particularly the earlier cases, were of questionable authenticity. After a careful review, 29 cases were selected on the basis of availability of an adequate history with histologic confirmation of the lesion. Nine cases were rejected because of the lack of histologic studies.[19] Two cases were omitted because of the lack of an adequate history.[20] Three cases were discarded because the lesions were either cancerous or were considered cancerous by the authors, although subsequent

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Traité de médicine, Paris, 1913, p. 176 (cited by Savy and others[11k]). (*e*) Barberio M.: Policlinico (sez. med.) **20**:37, 1913. (*f*) Savy, D.; Bonnet, P., and Martin, J. F.: Lyon chir. **9**:679, 1913. (*g*) Mayo, W. J.: Surg., Gynec. & Obst. **22**:1, 1916. (*h*) Grieg, D.: Edinburgh M. J. **27**:145, 1921. (*i*) Evans, A.: Brit. J. Surg. **9**:155, 1921. (*j*) Pickardt, E.: Klin. Wchnschr. **1**:1609, 1922. (*k*) Lepro diaz, J.: Bol. y trab. Soc. de cir. de Buenos Aires **8**:250, 1924. (*l*) Hall, I. W., and Borazil, W. H.: M. Chron. **6**:243, 1924. (*m*) Shapiro, P. F., and Lifvendahl, R. A.: Ann. Surg. **94**:61, 1931.

13. (*a*) Albers, J. F. H.: Atlas der pathologischen Anatomie für praktische Aerzte, Bonn, Henry & Cohen 1862, vol. 4, p. 490. (*b*) Holzinger, J.: Dissert., München, 1901; cited by Konjetzny.[11a]

14. (*a*) Bouisson, E. F.: De la bile, de ses variétés physiologiques, et de des altérations morbides, Paris, L. Castel, 1843, p. 305. (*b*) Wardell: Lancet **2**:407, 1869. (*c*) Dieckman, cited by Devic and Gallavardin: Rev. de méd. mém. orig. et bibliog. méd. **21**:570, 1901. (*d*) Ehrmann, C. H.: Musée de la Faculté de médicine de Strasbourg, Strasbourg, Berger-Levrault, 1843, p. 1.

15. (*a*) Husseinoff, D.: Zentralbl. f. allg. Path. u. path. Anat. **43**:344, 1928. (*b*) Shapiro and Lifvendahl.[12m] (*c*) Comfort, M. W., and Walters, W.: Ann. Surg. **93**:1142, 1931.

16. (*a*) Hammesfahr, C.: Zentralbl. f. Chir. **55**:3157, 1928. (*b*) Robson, A. W. M., and Dobson, J. F.: Diseases of the Gall-Bladder and Bile-Ducts, ed. 3, London, Baillière, Tindall & Cox, 1904, p. 200.

17. Duval, C. W.: J. Exper. Med. **10**:465, 1908.

18. Brentano, A.: Zentralbl. f. Chir. **47**:547, 1920.

19. de la Flor and Baraco.[6] Pozzi.[11a] Monari.[11f] McPhedran.[11g] Wilks and Moxon.[11i] Albers.[13a] Bouisson.[14a] Wardell.[14b] Dieckman.[14c]

20. Henry.[7] Marshall.[11q]

reviews included them in the benign category.[21] Two cases, in which the benign tumors were both incidental findings in patients with cancers of the ampulla of Vater and the duodenum, respectively, were rejected for the reasons discussed under papilloma and polyp.[22] Another case was discarded because of the admission of the authors that only the superficial portion of the neoplasm, a papilloma, had been studied histologically, and there still existed the possibility of a cancerous growth at the base of the papilloma.[5] One case was disqualified because the tumor originated from the duodenal mucosa rather than from the biliary tract.[11l] Two cases were discarded because the lesion arose from the gallbladder.[23] The original report of 1 case was not available, and in referring to this case Konjetzny [11a] failed to specify whether the tumor had been studied histologically. Finally, 4 cases—a case of melanoma of Vater's diverticulum, a case of carcinoid, and 2 cases of granuloma—were discarded for the reasons discussed in subsequent paragraphs.

Papilloma and Polyp.—The majority of the reported benign neoplasms involving the extrahepatic biliary ducts were epithelial tumors. Of these, papilloma and polyp are the most common, with a total of 24 cases recorded in the literature.[24] Only 10 of these cases, however, have been found acceptable in the present study.[25]

Papilloma and polyp are most commonly found in the common bile duct; in 9 of the 10 cases the tumor was found to have originated from that site. In the remaining case it originated from the hepatic duct near the point where the latter entered into the common bile duct. Infrequently, the tumor may extend for several centimeters along a duct or may even involve a large segment of the duct.

Generally, both papilloma and polyp assume only a moderate size, measuring at most several centimeters in greatest diameter. Occasionally, the tumor may obstruct the biliary outflow and produce a tremendous cystic dilatation of the duct, measuring up to a size of 11 by 10 by 7 cm. as in a case reported by Eve.[11j] The largest recorded growth, however, was a massive pedunculated papilloma, weighing 750 Gm., observed by Leriche [4] in a 4 year old child. This tumor was found implanted on the wall of the common bile duct.

Grossly, the tumors may appear as firm, elevated masses or as soft, vascular, sessile or pedunculated growths projecting into the ductal

21. Menetrier.[12d] Chappet.[11d] Sommer.[11a]

22. Meade.[9] Krause.[11b]

23. Savy, Bonnet and Martin.[12f] Jourdan.[11e]

24. These 24 cases comprise those cited in footnotes 4, 5, 6, 7, 8, 9 and all those in footnote 11.

25. These 10 cases comprise those cited in footnotes 4, 8 and 11b, e, j, k, m, o, p and r.

lumen. Histologically, the tumors are composed of thick or delicate papillae covered by a layer of tall columnar epithelium and supported by a fibrous or loose connective tissue core. This connective tissue core may be very vascular or, in some cases, may show mucoid degeneration.

Among the cases of papilloma and polyp discarded in the present study there were several in which these growths were associated with carcinoma of the ampulla of Vater, the common bile duct or the duodenum. These cases were rejected because frequently in cases of carcinoma of a hollow viscus, redundancy of the mucosa (due to the cancerous process) may produce papillomatous or polypoid growths of the epithelial surface. Or, as frequently occurs in the intestinal tract and the urinary bladder, cancerous proliferation may be present at the base of the papilloma which would not be detected if only the superficial portion of the tumor were removed. A case in point is that reported by Meade [9] in which a villous papilloma at the ampulla was excised together with the end of the common bile duct. Subsequent histologic studies revealed redundancy of the mucosa which assumed a villous papillomatous appearance. Nearby, however, a mass of cancerous spheroid cells was noted without any direct continuity with the papilloma. Bazin,[11p] in this connection, expressed the opinion that papilloma is potentially a cancer and warned that it should be so considered.

In their discussion of benign and cancerous extrahepatic bile duct tumors, Savy and associates [12f] expressed their belief that two conditions, inflammation and cholelithiasis, predisposed to benign tumor formation. In only 2 of the 10 cases in the present series did the observer state specifically that stones were found in the biliary system. In a case reported by Rolleston [11o] a papilloma was observed at the site of an impacted stone in the common bile duct. In a case reported by Pallasse [11m] the gallbladder and the common bile duct contained several calculi.

Adenoma.—Adenoma is second to papilloma and polyp in frequency of occurrence. Eighteen cases are reported in the literature,[26] 16 of which have been found acceptable for this study.[27] These include solid, cystic and mixed types. The mixed type includes tumors of glandular elements in combination with either muscle or fibrous connective tissue, which have been reported as adenomyoma and adenofibroma.

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26. These 18 cases comprise those cited in footnotes 3, 10 and 12.

27. The 16 acceptable cases comprise those cited in footnotes 3, 10 and 12a, b, c, e, f, g, h, i, j, k, l and m.

In 6 of the recorded cases the tumor arose from the common bile duct, near or at the ampulla of Vater. In another it was located at the junction of the cystic and hepatic ducts, and in 2 others it was reported as arising from the common hepatic duct. In 2 cases the tumor was described as situated in the remaining portion of the cystic duct subsequent to cholecystectomy. In 3 cases the tumor was of such considerable size that it was difficult to ascertain the site of origin. In 2 cases, reported by Keen [12a] and Hall and Borazil,[12i] the tumor was situated on the inferior surface of the right lobe of the liver and was believed to have originated from persistent remains of the vasa aberrentia (large aberrant bile ducts) during the course of the embryonic development of the biliary system.

In diameter the tumors may vary from a few millimeters to several centimeters. The largest tumor, reported by Evans,[12i] measured approximately 15 cm. in greatest diameter and extended from the edge of the liver to the umbilicus. Another large adenoma, reported by Keen,[12a] measured 11.5 by 9 by 4.5 cm. and weighed 113 Gm. Generally, the smaller growths are of the solid and mixed types, while the larger ones are of the cystic or cystadenomatous variety.

Grossly, the solid and the mixed growth appear as a various-sized, firm, and well circumscribed tumor within the wall of the bile duct. Occasionally, the tumor may appear as a diffuse thickening of the ductal wall. The cystadenoma, on the other hand, appears as a large, smooth, round or oval tumor, and frequently it shows multiloculated cystic spaces containing thick, viscid fluid on cut section.

Histologically, the solid adenoma is composed of numerous epithelial acini arranged in a fibrous connective tissue stroma. The epithelial cells are tall columnar in character with light-staining cytoplasm and basal nuclei. The mixed adenoma consists of abundant muscle of fibrous connective tissue elements in which are disposed scattered epithelial acini, similar in appearance to those found in the solid variety. The cystic type consists of numerous cystic spaces lined by tall columnar or cuboidal epithelium, which may project into the lumen in many places to form papillary epithelial infoldings. Frequently, the cyst lumen is filled with mucoid material. The cyst wall is composed of dense fibrous connective tissue in which blood vessels and occasionally small groups of epithelial acini may be found.

Savy, Bonnet and Martin [12f] pointed out the possibility that these adenomatous growths may assume a myxomatous appearance due to mucous transformation and may consequently be mistaken for myxoma. Wilks and Moxon [11] reported such a "myxomatous" tumor occupying practically the entire abdomen in a 3 year old child.

Savy and associates further asserted that early cases of adenoma of the bile ducts were actually not such but cases of glandular hyperplasia, since they found frequent mention of stones being present in the bile duct with the tumor. Among the cases reviewed in this series there were only 2 in which reference was made to the presence of stones.[28]

Fibroma.—Fibroma may arise anywhere along the extrahepatic biliary tract. In the case reported in the literature by Albers [13a] it was situated in the wall of the common bile duct and in that reported by Holzinger [13b] it involved the hepatic duct. In the present case it involved the common hepatic duct, proximal to the junction of the common hepatic duct and the cystic duct. Unfortunately, there is no mention of any histologic studies in Albers' report, and Holzinger's report was not available. However, the case reported by Albers resembles in many respects the case herein recorded. The tumor was the size of a "bean" and caused obstruction of the biliary canal with resultant enormous dilatation of the gallbladder and all of the bile ducts.

The histologic appearance of fibroma of the extrahepatic biliary ducts is essentially the same as that of fibroma found in other parts of the body. In the present case the fibrocytes were arranged in interlacing bundles with a suggestive concentric pattern. Of particular interest, however, were the focal collections of lymphocytes and plasma cells in the subserosa adjacent to the tumor and the fibrous adhesions seen grossly between the hepatic and cystic ducts at the site of the tumor. A previous inflammatory lesion of the duct predisposing to the fibromatous growth is suggested by these findings, but this is not substantiated by the history of the short and fatal illness. Also of interest is the fact that no calculi were found in either the gallbladder or the bile ducts.

The firm consistency of fibromas arising in the biliary tract is not conducive to their reaching a massive size as in some of the other types of tumor, as obstruction occurs early, due to compression and obliteration of the ductal lumen. In the present case the neoplasm measured less than 1 cm. in its over-all diameter but, owing to its consistency, was capable of producing first intermittent and later continuous obstruction of the hepatic duct terminating with biliary cirrhosis, extensive hepatic damage and bile nephrosis.

Lipoma.—Three cases of lipomatous masses in the extrahepatic bile ducts producing symptoms of biliary retention and icterus are reported in the literature.[14] Of these, none was confirmed by histologic studies. However, a case reported by Wardell [14b] in a girl 3 years of age is of interest. Clinically, the child manifested the progressive jaundice, clay-colored stools and dark saffron urine characteristic of biliary obstruction. At postmortem examination a tumor was found at the junction of the

28. Christopher.[8] Savy, Bonnet and Martin.[12f]

cystic and common bile ducts which was of the size of a large "horse bean." It was a yellowish white, homogeneous, nonvascular growth which gave a greasy stain to paper.

Lipomas in the diaphragm, the submucosa of the stomach and the ileum have been reported in the literature,[29] so that lipomas arising from the extrahepatic bile ducts are not inconceivable. None of those reported to date, however, have had histologic confirmation.

Neuroma.—Amputation neuroma has been recorded in 3 instances in the literature. In each case the growth followed cholecystectomy. In the case reported by Husseinoff [15a] the neuroma was found at autopsy in the blind end of the common bile duct; five years prior to death the patient submitted to an appendectomy and cholecystectomy, which was subsequently complicated by a duodenal fistula. In another case [15b] the new growth was an incidental finding at postmortem examination of a patient who died from cardiac disease but who had submitted to cholecystectomy fifteen years prior to death. Comfort and Walters [15c] reported a third case, in which symptoms of biliary obstruction developed ten years after cholecystectomy and appendectomy. Surgical intervention revealed a solid lesion in the middle portion of the common bile duct, measuring 1 cm. in diameter and completely obstructing the duct.

These 3 cases illustrate the fact that surgical procedures involving the gallbladder and bile ducts are not unattended by dangers and complications. Conversely, the infrequency of neuroma complicating any of the numerous surgical operations on the biliary system also reflects the care and technic exercised in modern surgery.

In the strictest sense, amputation neuroma is not a true neoplasm but rather a reactive hyperplasia. In view of the general acceptance of this lesion in most classifications of benign neoplasms, however, these cases have been included in this series. All of them have been confirmed by histologic studies.

Generally, amputation neuroma involving the biliary tract does not reach a substantial size. Like the fibroma, it produces obstructive manifestations on reaching a diameter of 1 cm. or more. Grossly, it may appear as a firm bulbous mass or a dense irregular mass of tissue embedding the blind end of a bile duct. Histologically, it is identical with amputation or traumatic neuroma found elsewhere.

Granuloma.—In earlier reviews, granuloma has been included among the benign tumors of the extrahepatic biliary ducts. Pathologically, in the strict sense, it is the result of an inflammatory process rather than

29. Botsford, T. W., and Newton, F. C.: Surgery **10**:265, 1941. Ballon, H. C., and Spector, L.: Canad. M. A. J. **41**:487, 1939. Rumold, M. J: Surgery **10**:242, 1941.

neoplasia. For this reason, 2 such cases[16] involving the bile ducts are not included here. One of the reported cases followed operation on the bile ducts with retention of suture material and resultant foreign body reaction. The other, reported by Robson and Dobson,[16b] lacked histopathologic details.

Melanoma.—One case of primary benign melanoma involving Vater's diverticulum and the lower portion of the common bile duct is reported by Duval.[17] A careful autopsy failed to reveal any primary site, and Duval postulated that the lesion arose from melanoblastic cells misplaced in the common bile duct. Although such a view is tenable, it is questionable that melanoma can arise as a primary tumor of the bile duct.

Laidlaw,[30] in his extensive studies on melanoma, found that nonectodermal mucous membranes normally harbor no melanoblasts, and he concluded that the occurrence of primary melanoma in nonectodermal tissue was highly improbable. Herbut and Manges,[31] reporting on cases of melanoma of the small intestine, applied the "dopa" reaction to the duodenum, the jejunum and the ileum without positive results. They likewise concluded that melanoma cannot arise in the small intestine as a primary tumor, although in 9 of the 25 cases which they reviewed in the literature the growth was reported as primary in the small intestine.

Embryologically, the common bile duct and the hepatic duct arise from the hepatic diverticulum which, in turn, is derived as a sacculation from the floor of the duodenum.[32] It follows, therefore, that since the bile ducts are of nonectodermal origin, primary melanoma of the bile ducts is highly improbable.

Finally, there is a further question as to the authenticity of the melanoma reported by Duval because occasional small vessels in the submucosa of the common bile duct and in close proximity to the tumor were reported to contain the pigment cells. These cells had a general morphologic aspect and were filled with dark brown granules similar to those of the cells described as constituting the tumor. This histologic observation is suggestive of two possibilities: The cells were either cancerous cells in blood vessels or phagocytic cells containing melanin-like pigments. Of the two, the latter is most probable, and consequently the tumor should be considered an adenomatous polyp with dark brown discoloration. This view is supported by the fact that the tumor was described by Duval as villous masses composed of glands

30. Laidlaw, G. F.: Am. J. Path **8**:477, 1932.

31. Herbut, P. A., and Manges, W. E.: Arch. Path. **39**:22, 1945.

32. Arey, L. B.: Developmental Anatomy: A Textbook and Laboratory Manual of Embryology, ed. 5, Philadelphia, W. B. Saunders Company, 1946, p. 226.

lined by a single layer of columnar cells that showed all grades of pigmentation. Unfortunately, the pigment was not identified, but it is quite possible that it may have been bile pigment accumulating in consequence of the chronic obstruction or a pigment similar to that found in melanosis coli as a result of protein disintegration due to the chronic obstruction.

Carcinoid.—Brentano [18] reported a case of carcinoid located retroduodenally near the ampulla in a patient 45 years of age. This was included by Christopher [9] in his review of benign neoplasms of the extrahepatic bile ducts. The case has been excluded from this report, however, because the author admitted that he could not ascertain the exact origin of the tumor and suspected that it arose from the duodenal papillae. He further expressed the view that the tumor could have arisen from the intestinal glands, misplaced Brunner's glands or from the pancreatic duct. Certainly, from available clinical data, carcinoids are most frequent in the appendix and small intestine, so that carcinoid originating from the common bile duct is the most remote possibility in this particular instance.

CLINICAL ASPECTS

Of the 30 cases of all types of benign tumor of the extrahepatic biliary ducts selected in this series, 18 concerned female and 9 male patients. In 3 cases the sex was not specified. The ages of the patients ranged from 4 years to 76 years. If 2 young children, whose tumors were most probably of congenital origin, are excluded, the adult patients ranged in age from 30 to 76 years, with an average age of 51 years.

Benign tumors have a tendency to remain dormant for long periods, and therefore the clinical manifestations in the early stages of the disease are vague and variable. Recurrent attacks of indigestion and flatulence, or vague pain in the right hypochondrium, or severe colicky pains in the epigastrium, which disappear after a few days, are some of the more commonly mentioned complaints. The duration of symptoms referable to the digestive or to the biliary system varies from a few weeks to eleven or twelve years.

Untreated patients invariably present in time the clinical manifestations of biliary obstruction. The onset of obstruction may be insidious, and the only complaint may be that of vague epigastric discomfort or insidious but progressive jaundice. In some cases the onset may be acute and associated with severe colic, nausea with or without vomiting, and intense jaundice. In either case, the course of illness culminates with severe colic, progressive and intense jaundice, nausea with or without vomiting, pruritus, clay-colored stools and dark saffron urine. As is evident, none of the symptoms are characteristic, as they may similarly be found with cholelithiasis or with a neoplasm involving the common bile duct, the gallbladder, the pancreas or the duodenum.

Physical findings are likewise nonspecific and may consist of enlargement of the liver, tenderness or presence of a mass on palpation over the right upper quadrant of the abdomen and generalized icterus.

Because of the lack of characteristic symptoms and physical findings, none of the reported cases was diagnosed preoperatively or ante mortem. In fact, 4 patients were completely symptomless, and the neoplasms were incidental findings at autopsy. Consequently, all authors are unanimous in the opinion that a correct clinical diagnosis is most difficult if not impossible. The rarity of the tumors certainly will not encourage one to venture a definite diagnosis. Nevertheless, such a possibility should be borne in mind, and surgical exploration is definitely indicated in the presence of signs and symptoms consistent with biliary duct obstruction. Furthermore, the possibility that a benign tumor, particularly adenoma or papilloma, may be associated with calculi in the bile ducts should also be considered. Finally, because of their benignity and tendency to remain dormant for long periods, these tumors should be attended by a favorable prognosis. In 11 of the 30 cases there was successful excision of the tumor, with recovery.

SUMMARY

Benign neoplasms of the extrahepatic ducts are rare. A review of the literature reveals 54 recorded cases, but in only 29 cases were there an adequate history and histologic confirmation. To these, an additional case is added. The lesions represented in the entire series of 30 cases are adenoma (16 cases), papilloma and polyp (10 cases), fibroma (1 case) and neuroma (3 cases).

The clinical manifestations are those of any obstruction to the extrahepatic bile ducts. In none of the recorded cases has a correct diagnosis been made preoperatively or ante mortem. Because of the inherent limited growth of the tumors, the prognosis should be good, and in the 30 accepted cases 11 patients underwent successful excision and recovery.

POSSIBLE ROLE OF INFECTION IN THE PRODUCTION OF PERIARTERITIS NODOSA IN HYPERTENSIVE RABBITS

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THE PROBLEM of mild hypertension and its relation to arteriosclerosis, arteriolosclerosis and periarteritis nodosa has been the subject of an enormous amount of speculation and experimental work. During a previous experiment [1] designed to permit investigation of the changes that occur in the thickness of the arterioles of the submucosa of the intestine of the rabbit in mild hypertension, several interesting observations were recorded. The rabbits never had a systolic blood pressure above 160 mm. of mercury, and yet there developed a measurable increase of the thickness of the media in the arterioles of the submucosa of the intestine. When these animals were compared with the control group, this increase was seen to be of statistical significance. Medial thickening was found also in the arterioles of the liver, the pancreas, the salivary glands and, rarely, the brain. In all situations the thickening was confined to the media and appeared to be the result of hyperplasia or hypertrophy of the musculature. In a small number of animals in which the clinical and chemical picture of uremia developed, lesions of arteriolonecrosis as opposed to arteriolosclerosis were found. These observations were in essential agreement with those of previous writers.[2]

However, 3 of these animals accidentally became infected during the operative procedure and subsequently arterial and arteriolar lesions developed which resembled those of periarteritis nodosa in man. Accordingly, it was decided to test the thesis that in the presence of mild hypertension infection may result in periarteritis nodosa.

METHODS

Twenty-two white rabbits were used. All were housed in the same quarters and allowed food and water ad libitum. Eight were kept as controls, and 6 were subjected to nephrectomy on the right and a wrapping of the left kidney in

From the Department of Pathology, Duke University School of Medicine.

1. Kipkie, G. F.: Unpublished data.

2. Goldblatt, H.; Lynch, J.; Hanzul, R., and Summerville, W. W.: J. Exper. Med. **59**:347, 1934. Goldblatt, H.: Ann. Int. Med. **11**:69, 1937; J. Exper. Med. **67**:809, 1938; Am. J. Clin. Path. **10**:40, 1940. Wilson, C., and Pickering, G. W.: Clin. Sc. **3**:343, 1938.

polythene cellophane after the technic of Page.[3] The pressure rises in this group were satisfactory, although two partial failures tended to lower the mean pressure level. The remaining 8 rabbits were subjected to a similar procedure except that a pledget of gauze soaked in a culture of Micrococcus albus (Staphylococcus albus) was inserted under the cellophane. In 2 of these animals an elevation of blood pressure failed to develop.

Determinations of blood pressure were done twice weekly on the central artery of the depilated ear by an indirect method with a pressure capsule as suggested by Grant and Rothschild.[4] Previous investigators have shown that the pressure levels obtained by this method are approximately 20 mm. of mercury lower than the actual pressures as obtained by cannulation of the carotid artery. This was substantiated by the carotid artery cannulations performed at the end of both the previous and the present experiment.

Once a week, all animals were bled from a right ear vein, and the creatinine level of the blood serum was determined. This level was used as an index of the retention of the waste products of protein metabolism and thus of renal function.

The control animals had a mean pressure of 62 mm., the noninfected hypertensive animals a mean pressure of 92 mm. and the infected hypertensive animals a mean pressure of 102 mm. of mercury. Although some of the animals showed disappointing elevations of pressure, it was felt they would indicate whether the cellophane alone could be held responsible for any changes that might be observed. None of the animals presented clinical or laboratory signs of uremia.

The total duration of the experiment was one hundred and two days. Zenker's solution was used as the routine fixative. Brains were fixed in formaldehyde-saline solution and the eyes in Bouin's solution. Sections were cut at 6 microns and stained with hematoxylin and eosin. No tissue in the body except the spinal cord was omitted from sectioning.

RESULTS

In all cases in which the procedure was one of operation and infection, M. albus (Staph. albus) was recovered from the peritoneal cavity and from beneath the cellophane capsule. Escherichia coli was obtained in 1 instance. Blood was not obtained for culture.

No changes were seen in the arterioles of any of the control animals (fig. 1*A*). The hypertensive animals without infection showed thickening of arterioles similar to that observed in the previous experiment (fig. 1*B*). These arteriolar changes were found chiefly in the submucosa of the intestine although they were noted in the liver, the pancreas, the salivary glands and rarely the skeletal muscle. They ranged from mural thickening or swelling confined to the media to lesions in which increased numbers of nuclei were present. In the hypertensive group with infection, 2 of 6 animals showed vascular changes which were different from the lesions noted in the noninfected group.

The lesions in the hypertensive, infected group showed varying stages of development in each animal. The earliest recognizable change appeared to be a swelling of the muscle cells of the media or perhaps just a homogeneity of the medial tunic. In some arterioles only a small

3. Page, I. H.: Science **89**:273, 1939; J. A. M. A. **113**:2046, 1939.
4. Grant, R. T., and Rothschild, P.: J. Physiol. **81**:265, 1934.

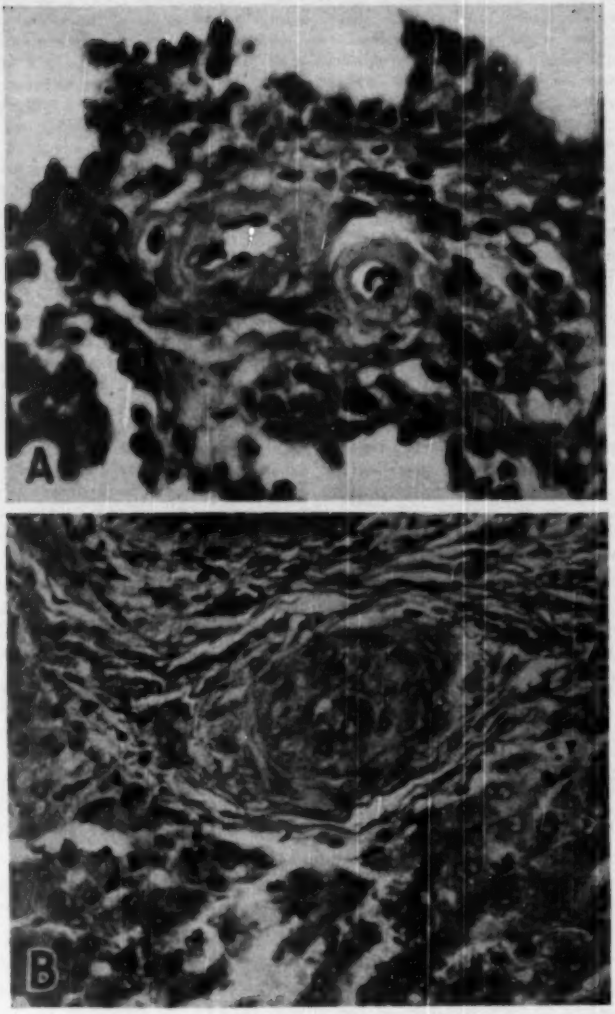

Fig. 1.—*A*, arteriole of the submucosa of the intestine of a normal rabbit; hematoxylin and eosin; × 325. *B*, arteriole in portal space of the liver of a hypertensive rabbit showing thickened media; hematoxylin and eosin; × 325.

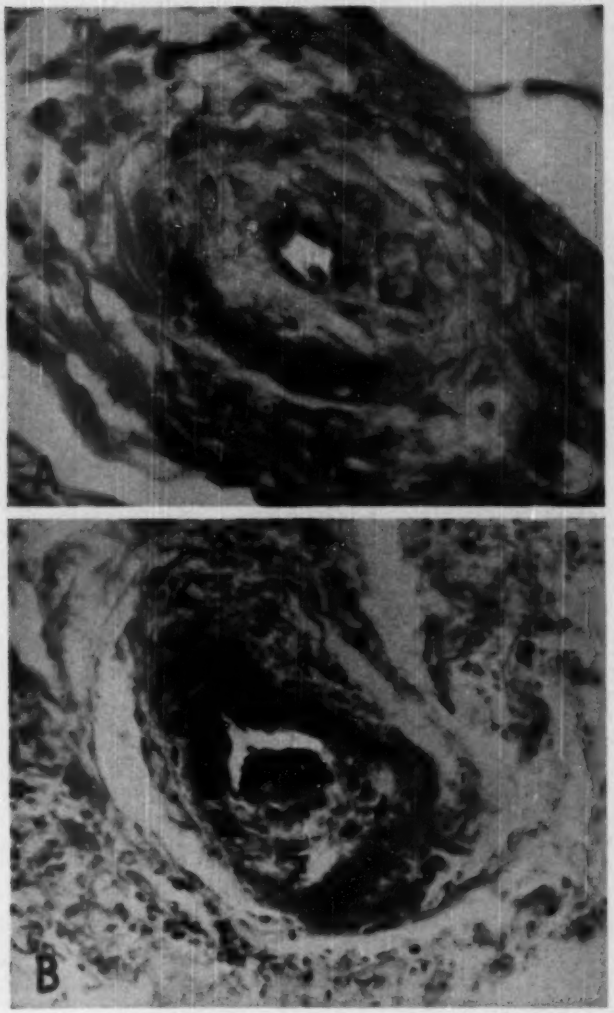

Fig. 2.—*A*, segmental involvement of the media of an arteriole of a hypertensive, infected rabbit; hematoxylin and eosin; × 900. *B*, involvement of the entire circumference of a vessel of a hypertensive infected rabbit; hematoxylin and eosin; × 325.

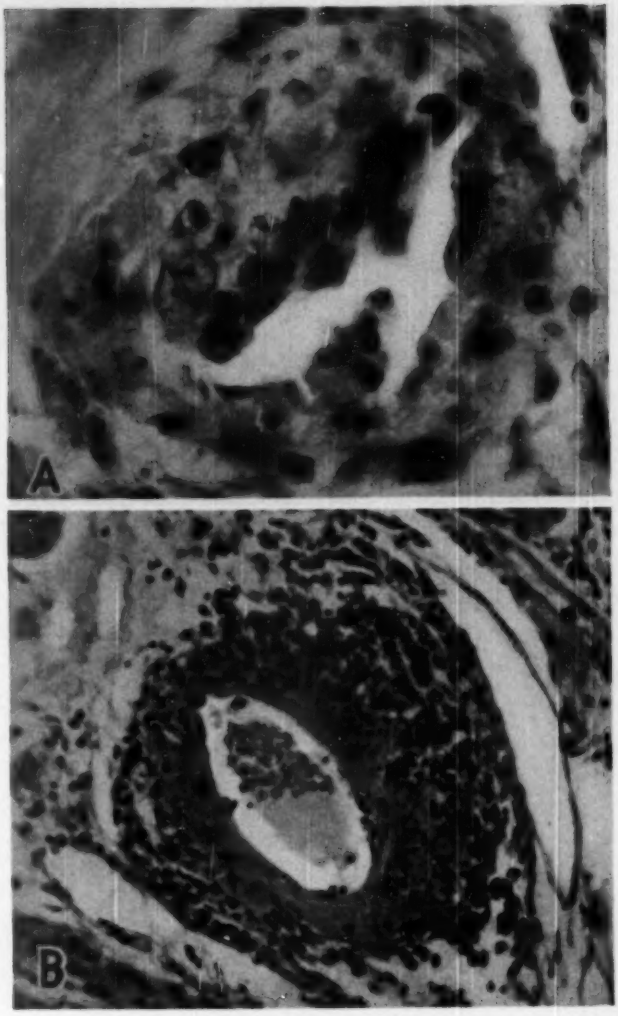

Fig. 3.—*A*, varied cellular infiltration of involved arteriole of a hypertensive, infected rabbit; hematoxylin and eosin; × 900. *B*, lesion resembling periarteritis nodosa in a hypertensive, infected rabbit; hematoxylin and eosin; × 325.

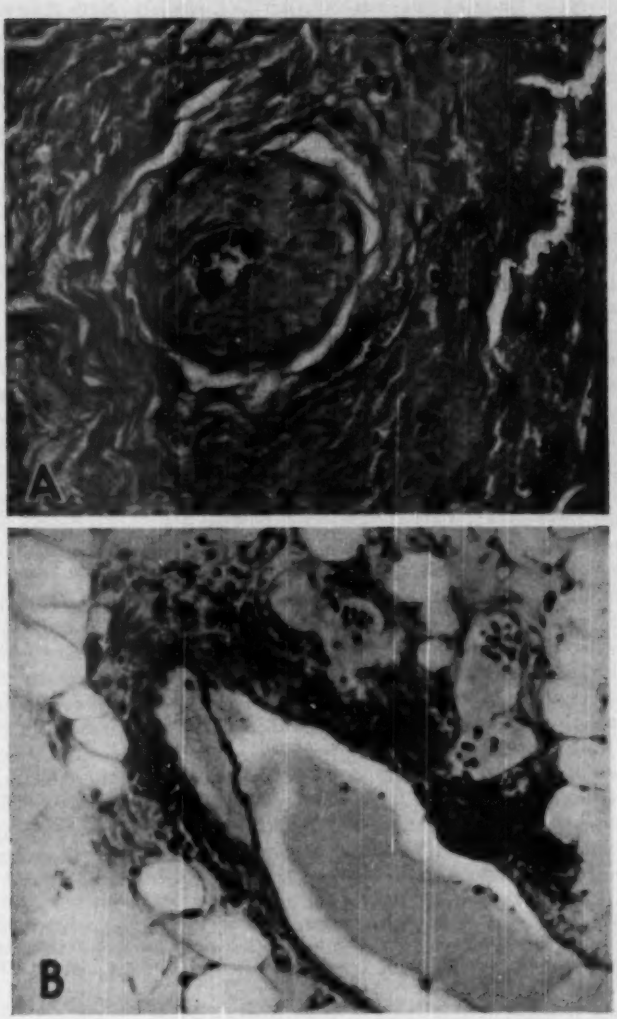

Fig. 4.—*A*, old lesion showing fibrosis and narrowing of lumen in a hypertensive, infected rabbit; hematoxylin and eosin; × 325. *B*, involvement of a segment of a vein in a hypertensive, infected rabbit; hematoxylin and eosin; × 150.

segment of the vessel was involved (fig. 2*A*), while in others the entire circumference showed changes (fig. 2*B*). The next stage appeared to be hemorrhage involving the media or the adventitia or both layers. This gave the appearance of "fibrinoid change" in the vessels and became granular in character and eosinophilic in staining capacity. The cellular infiltration varied considerably (fig. 3*A*). In some, a few polymorphonuclear leukocytes and macrophages could be found only at one pole of the vessel, while in others, polymorphonuclear leukocytes and eosinophilic cells were present in such numbers that it was impossible (fig. 3*B*) to determine exactly the limits of any of the layers of the vessel wall. In lesions which appeared to be slightly older, numerous macrophages were found at the periphery. Old lesions showed fibrosed arteriolar walls with narrowed lumens (fig. 4*A*). The vessels involved were of the caliber of small arteries and arterioles. The intestine and rarely the liver were the sites of vascular change. Rarely a vein or a venule appeared involved in a similar fashion (fig. 4*B*). Intravascular thrombosis was fairly common, but no recanalization of any of the thrombosed vessels was seen.

COMMENT

It is clear that definite conclusions are not justified from the present experiments, but certain speculation is possible.

It is known that lesions resembling periarteritis nodosa have been produced experimentally in rabbits by the injection of horse serum [5] and that many animals into which horse serum has been injected have glomerulonephritis.[6] Wilson and Bryom [7] noted periarteritis in rats, due to clamping the renal artery, and felt that the determining factor was the sudden strain imposed on the vessel wall by the vasoconstriction and the resultant rise in blood pressure. The rats in which Cromartie [8] observed the development of periarteritis were all hypertensive, but not all had perirenal infection. Hypertension is usually found in human cases of periarteritis nodosa, and the lesions have been found in patients who manifested hypersensitive reaction to serums or sulfonamides. It must be remembered that not all patients having periarteritis nodosa are hypertensive. Also, it must not be forgotten that a lesion resembling periarteritis develops in many rats spontaneously, particularly if they are more than 400 days old.

5. (*a*) Hopps, H. C., and Wissler, R. W.: J. Lab. & Clin. Med. **31**:939, 1946. (*b*) More, R. H., and McLean, C. S.: Am. J. Path. **25**:413, 1949. (*c*) Rich, A. R., and Gregory, J. B.: Bull. Johns Hopkins Hosp. **72**:65, 1943.

6. Cavelti, P. A., and Cavelti, E. S.: Arch. Path. **40**:158 and 163, 1945. Masugi, M.: Beitr. z. path. Anat. u. z. allg. Path. **92**:429, 1934. More, R. H., and Waugh, O.: J. Exper. Med. **89**:541, 1949. Rich and Gregory.[5c]

7. Wilson, C., and Bryom, F. B.: Lancet **1**:136, 1939.

8. Cromartie, W. J.: Am. J. M. Sc. **206**:66, 1943.

Smith, Zeek and McGuire[9] found periarteritis nodosa in 40 per cent of rats made hypertensive by wrapping the kidneys in silk and noted that the periarteritic animals had a higher mean monthly blood pressure than the animals in which no periarteritis nodosa was found.

Later, Smith and Zeek[10] produced lesions of periarteritis in rats by a unilateral nephrectomy and wrapping of the remaining kidney in silk contaminated with staphylococci or salmonellas or with material obtained by rubbing the silk on the floor. They were unable to produce periarteritis in rabbits by injecting horse serum into sensitized animals. They concluded that the data supported the hypothesis that periarteritis nodosa was associated with sharply rising hypertension and tended to refute the theory that the condition was the result of hypersensitivity to known or unknown antigens.

Recently Smith, Zeek and Weeter[11] attempted to define what they called "periarteritis nodosa" of man and animals and "hypersensitive angeitis" of man. In the rat the "periarteritic" lesions first appeared on the second or third day following nephrectomy and wrapping of the remaining kidney in silk. They consisted of fragmentation, degeneration and focal edema of adventitial collagen at the bifurcation of arteries of the muscular type. This was followed by proliferation of fibroblasts and histiocytes. These occasionally surrounded the vessels and spread from points of origin in the media to the adventitia. Necrosis and inflammatory exudation appeared between the seventh and the eleventh day. It began in the outer media and adventitia and rarely was circumferential. The inflammatory cells were pleomorphic, with eosinophilic cells and foreign body giant cells appearing. Thrombosis and aneurysmal dilatation occurred. Stages 4 and 5 showed healing by development of granulation tissue, with vascular fibrous tissue being produced near the bifurcation of muscular arteries. The higher the pressure, the more likely was the occurrence of periarteritis nodosa.

"Hypersensitive angeitis" of man was similar to the third stage of the periarteritic lesions except that the fibroblasts and new capillaries were absent, and thus no granulation tissue was found around the vessel wall. The lesions of this type were in the arterioles and small arteries of viscera including the lung. They were common in the walls of portal veins. Fibrinoid necrosis was found in veins also. A trabeculitis of the spleen and a necrotizing glomerulonephritis were present in these cases, and there was no evidence of healing.

Periarteritis may be caused by hypersensitivity and even in the cases of pure hypertension the foreign protein usually introduced to produce the hypertension may have provided the sensitizing protein.

9. Smith, C. C.; Zeek, P. M., and McGuire, J.: Am. J. Path. **20**:721, 1944.
10. Smith, C. C., and Zeek, P. M.: Am. J. Path. **23**:147, 1947.
11. Smith, C. C.; Zeek, P. M., and Weeter, J. C.: Am. J. Path. **24**:889, 1948.

Smith and Zeek [10] have suggested that in rats periarteritis nodosa is not the result of the hypertension but is a frequent complication whenever the method used in producing hypertension results in a sharply rising blood pressure curve with peaks averaging between 200 and 240 mm. of mercury or higher.

While the drawing of definite conclusions from the present experiments is not justified, it is interesting to note that 2 animals of the infected group had terminal carotid artery cannulation pressures above the level of the 2 showing periarteritis nodosa and that 2 had approximately the same pressure. None of these pressures was above 178 mm. of mercury. The blood pressure of the 2 animals showing periarteritis nodosa was 154 and 130 mm., respectively. Thus, in the rabbit, pressures above 200 mm. of mercury do not seem necessary for the production of periarteritis nodosa. That the animals might have been sensitive to the polythene cellophane used in wrapping would seem to be disproved by the fact that none of the rabbits in this or a previous experiment showed lesions of periarteritis, whether hypertension developed or not, unless they were infected. Also, infection alone does not seem sufficient to cause the lesions since nonhypertensive animals, although subjected to the same operative procedure as that described with infection, did not subsequently reveal periarteritis nodosa.

When the lesions herein reported are examined carefully, they seem to fit into the periarteritis nodosa type instead of the "hypersensitive angeitis" type of Smith, Zeek and Weeter.[11] They showed fibroblastic and histiocytic responses as well as the typical pleomorphic cellular infiltration although no giant cells were seen. They appeared to heal by the formation of granulation tissue. The picture of fragmentation, degeneration and focal edema of adventitial collagen was not observed, since no animal was killed during the early stage when these changes are observed.

Localizing of lesions at the bifurcation of muscular arteries was rarely seen. Instead it was noted that the arterioles and small arteries were chiefly affected. The portal veins and hepatic arteries were involved, but in none of the cases did one see the trabeculitis of the spleen or the necrotizing glomerulonephritis found in the "hypersensitive angeitis."

Lesions were uniformly absent from the hearts of both the infected and the noninfected hypertensive animals, in contrast to the high incidence of coronary artery involvement in periarteritis nodosa in man.

The wrapped kidney showed an absence of lesions other than the perinephric fibrous connective tissue. An occasional animal had calcific deposits in the apexes of the pyramids and nephroliths in the urinary pelvis, these findings being practically unknown in the rabbit in its normal habitat.

An attempt was made to determine whether or not hypertrophy of the smooth muscle cells of the juxtaglomerular apparatus of the kidney [12] had occurred. Although there seemed to be a greater than normal number of nuclei present in the macula densa, no definite conclusions can be drawn at this time.

Experiments are now in progress to increase the series of hypertensive, infected rabbits and also to test the effect of injection of the products of the growth of M. albus used in this experiment as a means of evaluating the sensitivity feature of periarteritis nodosa in hypertensive rabbits. The causes of periarteritis nodosa have not been demonstrated by this experiment, but it does seem that hypertension of a moderate or severe degree is a necessary feature. The secondary feature necessary for the production of periarteritis nodosa, whether infection or hypersensitivity, is not clearly apparent at this time.

SUMMARY

From this experiment it appears that the periarteritis nodosa produced in the white rabbit is dependent on several factors, one of which is a moderate degree of elevation of the blood pressure.

It is suggested that infection may be the secondary factor, although it has not been possible to rule out hypersensitivity as a precipitating feature.

12. Dunihue, F. W., and Candon, B. H.: Arch. Path. **29**:777, 1940. Dunihue, F. W.: Ibid. **32**:211, 1941. Goormaghtigh, N., and Grimson, K. S.: Proc. Soc. Exper. Biol. & Med. **42**:227, 1939. Goormaghtigh, N.: Am. J. Path. **16**:409, 1940.

DEMYELINATION IN THE GUINEA PIG IN CHRONIC ALLERGIC ENCEPHALOMYELITIS

Produced by Injecting Guinea Pig Brain in Oil Emulsion Containing a Variant of Mycobacterium Butyricum

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RIVERS, Sprunt and Berry [1] discovered that acute disseminated encephalomyelitis can be produced in monkeys by often repeated intramuscular injections of emulsions and alcohol-ether extracts of rabbit brain. Later, Rivers and Schwentker [2] extended these observations. One characteristic aspect of this experimental disease was its acute nature. It was found that once the neurologic signs and symptoms appeared, the disease progressed fast and the monkeys were put to death in two to three weeks after the onset of symptoms. The histologic changes in the brain were at first perivascular; proliferation of glial elements, infiltration of mononuclear cells and polymorphonuclear eosinophilic leukocytes were conspicuous. Demyelination occurred mainly about the blood vessels but was not altogether confined to them.

Rivers and his associates emphasized the similarity of the pathologic changes observed in the experimental acute disseminated encephalomyelitis and the changes described in man following jennerian prophylaxis, smallpox, measles and antirabic vaccination. They, however, also observed in their preparations multinucleated elements recalling a finding in a chronic malady, namely, progressive subcortical encephalopathy (Schilder's disease). These results were confirmed by Ferraro and Jervis.[3]

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From the Public Health Research Institute of the City of New York, Inc. (Drs. Freund and Lipton) and the Department of Neuropathology of Harvard Medical School and the Massachusetts General Hospital (Dr. Morrison), Boston.

1. Rivers, T. M.; Sprunt, D. H., and Berry, G. P.: J. Exper. Med. **58**:39, 1933.

2. Rivers, T. M., and Schwentker, F. F.: J. Exper. Med. **61**:689, 1935.

3. Ferraro, A., and Jervis, G. A.: Arch. Neurol. & Psychiat. **43**:195, 1940.

The original purpose of these authors was to throw light on the causation of encephalitis occurring rarely in man subsequent to certain infectious diseases and antirabic vaccination. The workers [3] who took up this subject thereafter were interested in certain aspects of the experimental disease which are also found in chronic neurologic maladies such as multiple sclerosis and progressive subcortical encephalopathy. The lesions produced in monkeys receiving numerous injections of brain over a period of months or a single injection of brain or cord in a water-in-oil emulsion containing killed mycobacteria are almost identical except that foreign body giant cells were observed in the former. The aim of some recent studies was to produce the experimental disease in chronic form with conspicuous demyelination. Ferraro and Cazullo [4] reported recently a successful attempt in this direction. Monkeys were given repeated injections of brain and adjuvants, with smaller amounts of brain and killed tubercle bacilli being employed than were used by others and by themselves in previous experiments.

We encountered a chronic demyelinating form of encephalomyelitis in the guinea pigs after a single injection of homologous brain in water-in-oil emulsion containing a killed variant of Mycobacterium butyricum hitherto not used in similar experiments. With few exceptions the demyelination thus far seen in this species of animals was limited to the perivascular spaces. Alvord and Stevenson,[5] as well as Lumsden,[6] however, occasionally observed demyelination that was more extensive than the narrow sleeve of myelin loss so commonly reported.

The experiments which led to the observations to be described were designed to study the mode of action of mycobacteria in altering the process of sensitization to unrelated antigens. One or a few injections of brain emulsified in paraffin oil do not induce allergic encephalomyelitis [7] unless killed Mycobacterium tuberculosis or Myco. butyricum or Nocardia asteroides [8] is added to the emulsion. We have also produced allergic encephalitis in 1 of 15 guinea pigs substituting killed Coccidioides immitis for mycobacteria. Brewers' yeast and lycopodium powder were ineffective. Since the strain of N. asteroides used was acid fast, the question rose whether cells which are not acid fast and

4. Ferraro, A., and Cazullo, C. L.: J. Neuropath. & Exper. Neurol. **7**:235, 1948.

5. Alvord, E. C., Jr., and Stevenson, L. D.: Experimental Production of Encephalomyelitis in Guinea Pigs, A. Research Nerv. & Ment. Dis., Proc. **28**:99, 1950.

6. Lumsden, C. E.: Brain **72**:198, 1949.

7. Freund, J.; Stern, E. R., and Pisani, T. M.: J. Immunol. **57**:179, 1947.

8. Freund, J., and Lipton, M. M.: Proc. Soc. Exper. Biol. & Med. **68**:373, 1948.

are less rich in lipids could act as potentiating agents.[9] Long and Finner [10] have shown that the lipid content of tubercle bacilli is related to the glycerin content of the culture medium when Long's medium is used. Accordingly, Myco. butyricum was grown on beef heart agar in the absence of glycerin. The cells grown on this medium were found

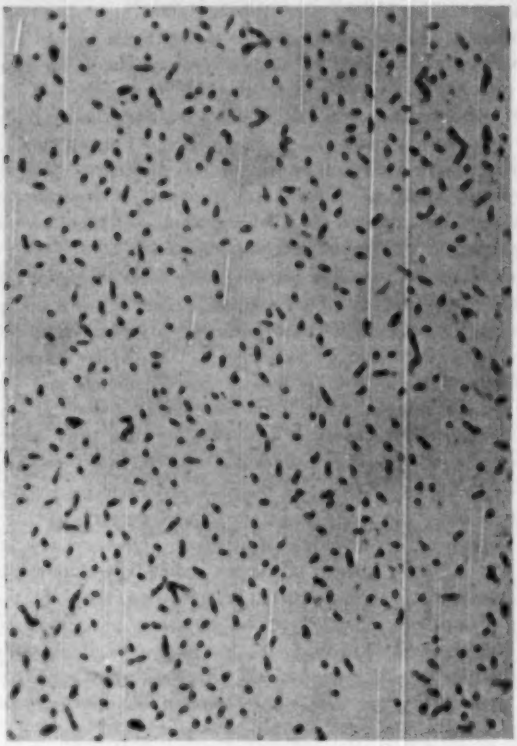

Fig. 1.—Myco. butyricum grown on beef heart agar in the absence of glycerin; × 1,900.

to be different from those grown on Long's medium containing 5 per cent glycerin. The majority of cells on the former medium are not

9. Mycobacterium "timothy bacillus" (or Mycobacterium phlei) acted like Myco. tuberculosis in altering horse serum sensitization (Freund, J., and Walter, A. W.: Proc. Soc. Exper. Biol. & Med. **56**:47, 1944).

10. Long, E. R., and Finner, L. L.: Am. Rev. Tuberc. **16**:523, 1927.

acid fast and not alcohol fast. There are also morphologic differences. The bacteria grown without glycerin are shorter, and many of them are almost coccoid (fig. 1); on a touch preparation their distribution seems haphazard in contrast to that of cells grown on Long's medium. On the latter medium they are characteristically acid and alcohol fast

Fig. 2.—Myco. butyricum grown on Long's medium; × 1,900.

and a touch preparation shows that they are oriented (fig. 2). Two or three rods may be found close together and in parallel position, almost touching two similar groups of two or three rods, sometimes at obtuse angles, as if they were parts of a filament. Some of these filamentous structures in the preparation cross each other in a haphazard fashion.

METHODS AND MATERIALS

The strain of Myco. butyricum used for the experiments was grown on Long's medium and transferred to beef heart agar, and was subcultured four times on this medium. The growth was suspended in distilled water containing formaldehyde solution U. S. P. in a concentration of 0.1 per cent. After one hour the fluid was removed, and, to aid the drying, the cells were suspended in cold acetone for five minutes. The acetone was discarded, and the cells were dried under reduced pressure over sulfuric acid. Each guinea pig received 2.5 cc. of an emulsion containing 1 cc. of 10 per cent guinea pig brain suspension in isotonic sodium chloride solution, 1 mg. of Myco. butyricum suspended in 1 cc. of liquid petrolatum [11a] and 0.5 cc. of a commercial absorption base.[11b] The technical details of the preparation of the emulsion may be found in previous papers.[12] The emulsion was injected into the subcutaneous tissue of the back of the neck in three sites. The local reactions as judged by palpitation were not different from those usually seen after the injection of a similar material with Myco. butyricum grown on a medium containing glycerin.

Stains.—The brains and spinal cords were fixed in formaldehyde solution U. S. P. diluted 1:10, and the following stains were used: cresyl violet, hematoxylin and eosin, Van Gieson, Weil, Weigert, Spielmeyer, oil red O, black sudan B, Holzer, Masson, Cajal's silver for axons, Hortega's silver for astrocytes and Foot's silver for reticulum.

EXPERIMENTAL RESULTS

Two separate experiments were carried out. In the first experiment 11 guinea pigs were used. Of these, 2 had no symptoms for six months, and no lesions were found in the central nervous system by histologic examination. In 9 guinea pigs paralysis of the hindlegs and characteristic lesions of the central nervous system developed. In 6 of these, paresis or paralysis of the hindlegs appeared within sixteen to twenty-two days after injection. Within six days after the onset of the symptoms the animals became moribund and were killed. The lesions of the central nervous system were found to be similar to those described in the preceding paper and included perivascular cuffing, ependymal thickening, focal accumulation of mononuclear cells, thrombi and microscopic hemorrhages.[7] In 3 guinea pigs the disease took a chronic form. One of these was killed two months after the onset of symptoms when it was near death. The other 2 had shown symptoms for one hundred and seventy-one and one hundred and ninety days, respectively, when they were killed for histologic examination. In these animals the lesions of the central nervous system differed from

11. (*a*) Bayol F® was used; it is described as a paraffin oil of low viscosity. It may be obtained from Stanco Distributors, New York. (*b*) Falba® was used. It is said to be a mixture of oxycholesterol and cholesterols derived from hydrous wool fat manufactured by Pfaltz & Bauer, Inc., New York.

12. Freund, J.; Thomson, K. J.; Hough, H. B.; Sommer, H. E., and Pisani, T. M.: J. Immunol. **60**:383, 1948.

those in guinea pigs with the acute form of the disease. The description of the lesions is given in the following section.

In the second experiment 10 guinea pigs were used. One of these had shown no symptoms for seven months when it was killed for histologic examination; no lesions were found in the central nervous system. Another guinea pig had no symptoms for sixty-six days. It died during pregnancy, and no lesions were seen in the central nervous system.

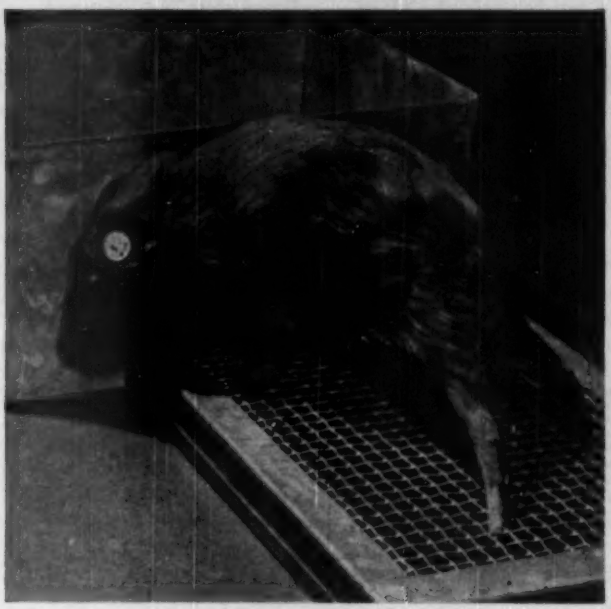

Fig. 3 (experiment 31, guinea pig 3).—Spastic condition in later days of paralysis.

Of the 8 remaining animals, 7 had the disease in acute and 1 in chronic form. In the former group, the onset of symptoms occurred in eighteen to twenty-eight days after injection, and the symptoms lasted from three to twenty-five days. The animals were killed when moribund. The lesions of the central nervous system were the usual ones. In the last animal, however, the symptoms were first noticed thirty-two days after injection, and they had lasted for one hundred and seventy-one days when the guinea pig was killed for histologic examination.

OBSERVATIONS RESPECTING THE CHRONIC FORM OF ENCEPHALOMYELITIS

Clinical Findings.—In experiment 31, guinea pig 3 had flaccid paralysis of both hindlegs, noticed fifty-six days after injection. However, seventy-three days later it was found that the hindlegs were spastic (fig. 3), and they had remained spastic for one hundred and sixty-eight days when the animal was killed. The circumference of the hindlegs became diminished at about eighty days after injection. Urinary incontinence was noticed about two months after injection and lasted for about one month. During the course of the experiment, the guinea pig had been gaining weight for nine weeks when the symptoms appeared. During the following ten weeks it failed to gain. This

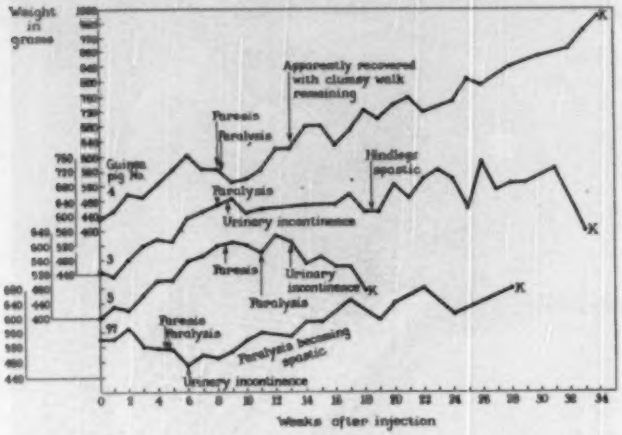

Fig. 4.—Clinical findings in guinea pigs at intervals after injection correlated with shifts in weight.

period was followed by a fluctuation of weight for twelve weeks. Subsequently, the animal lost weight rapidly (fig. 4).

In experiment 31, guinea pig 4 had paresis of the hindlegs fifty-six days and paralysis fifty-eight days after sensitization. The paralysis subsided slowly, and ninety days after injection the guinea pig had no symptoms other than a clumsy walk, which had been present up to two hundred and forty-six days after injection when it was killed for histologic examination. This animal lost weight coincidentally with the onset of paralysis but started to gain when the symptoms disappeared and was gaining weight throughout the period of observation.

In experiment 31, guinea pig 5 had no definite symptoms for sixty days when paresis of both hindlegs appeared; seventeen days later the right leg, and one day later both hindlegs, became paralyzed. Urinary

incontinence was observed ninety-one days after sensitization. The guinea pig remained in this condition until it was killed, i. e., one hundred and twenty-seven days after sensitization. The animal gradually became weaker and was killed because it appeared to us that it might not survive for more than one or two days. The body weight increased until the symptoms appeared; then it fluctuated for a few weeks and subsequently fell rapidly.

In experiment 40, guinea pig 97 showed paresis of both hindlegs within thirty-two days after injection. On the following day the legs were paralyzed. The paralysis persisted for one hundred and seventy-one days, the animal remaining vigorous until it was killed for histologic examination. The paralysis was flaccid for about two and a half months; then it gradually became spastic. Urinary incontinence was noticed forty-two days after sensitization. The animal started to lose weight during the third week just before the appearance of neurologic symptoms. It began to gain weight eight weeks after sensitization, and the gain was slow and fluctuating throughout the period of observation.

Histologic Observations.—These animals presented histologic alterations that were of the same general type in all cases. Lesions were present in a scattered way in the cerebrum, the cerebellum and the basal ganglions, while in the spinal cord they were more numerous and more severe. The lesions were chiefly present in the white matter and to a lesser extent in the gray. Among the more common sites were the following: corpus callosum, periventricular white matter, fimbria fornicis, chiasma opticum, tractus opticus, corpus restiforme and various parts of the spinal cord. The posterior and lateral columns of the spinal cord were more often involved than the anterior column. The lumbosacral region was more severely diseased than other regions, but the lesions of the thoracic portion of the cord were also extensive.

Meningitis was present in all cases and was usually of a focal nature. It was most conspicuous in guinea pig 5, in which it was present beneath the occipital lobe, among the folia of the cerebellum and surrounding the medulla and the spinal cord. The inflammatory exudate consisted mostly of lymphocytes and plasma cells, but some histiocytes were also present, and where these cells surrounded blood vessels in the subarachnoid space the exudate was several layers thick, thinning out as it receded. In animal 4 the meningeal reaction was limited to a few thick-walled subarachnoid vessels that were surrounded by dense collars of adventitial cells. No widespread areas were found in that animal. There was thickening of the meninges in cord and brain, but active meningitis had nearly subsided, only a few scattered foci of lymphocytes remaining over the frontal and occipital zones of the cortex, among the folia of the cerebellum and in the cervical-thoracic region

of the cord. The lumbosacral part of the cord showed thickened meninges but not much inflammation. Choroiditis and ependymitis were likewise present in all animals. In animal 3 a conspicuous lymphocytic infiltration was present throughout much of the choroid plexus in the lateral ventricles, while in the other animals localized perivascular cuffing was present near the base of the choroid. Beneath the ependyma vessels were surrounded by collars of hematogenous cells in the Virchow-Robin space.

The inflammatory exudate followed the blood vessels in from the subarachnoid space to the depths of the tissue, and this was seen especially in the spinal cord, where conspicuous parenchymatous lesions

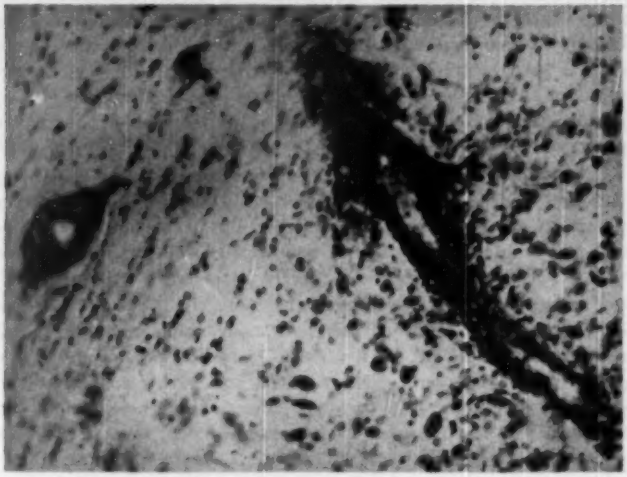

Fig. 5 (guinea pig 3).—Perivascular reaction of adventitial cells and lymphocytes, and in the parenchyma, microglia. Nissl stain; × 100.

were present in the white matter. The thickened blood vessel wall was often surrounded by layers of histiocytes or monocytes, the lymphocytes not being numerous in the perivascular space in the depths of the tissue. Beyond the Virchow-Robin space in the parenchymatous tissue there were foci of perivascular microglial hyperplasia. The microgliocytes were in various degrees of activation ranging from slightly swollen cells with spiky processes to fully formed gitter cells. This was the most characteristic feature of the disease and often the microglial hyperplasia surrounding a blood vesel would extend sufficiently far outward to blend with other foci surrounding other vessels so that a larger lesion resulted (fig. 5).

These confluent foci of reaction were present in the midst of zones of demyelination, as revealed by the myelin stains. When a single vessel was surrounded by a collar of reacting cells the loss of myelin was often not detectable, but when several of these perivascular foci merged, the larger lesion resulting showed a conspicuous absence of myelin (see figs. 6 and 7). Any of the conventional myelin stains (Weigert, Weil, Spielmeyer) showed up the myelin defect. It was

Fig. 6 (guinea pig 3).—Transverse section of the spinal cord. Note the patch of demyelination on the edge of the lateral column. Weigert's stain; × 30.

also clearly seen in the black sudan B stain. But in the black sudan B stain there was no fat in the demyelinated zone, nor was any fat detected by means of oil red O. In guinea pig 97 there were lesions especially in the pons and the medulla and conspicuous foci in the brachium conjunctivum, where microglial hyperplasia was seen perivascularly and in the spinal cord, in a peripheral, subpial location. In the thoracic portion of the cord this was especially conspicuous. Perivascular demyelination was present in small scattered foci, but larger patches were present in the posterior column of the cervical and thoracic

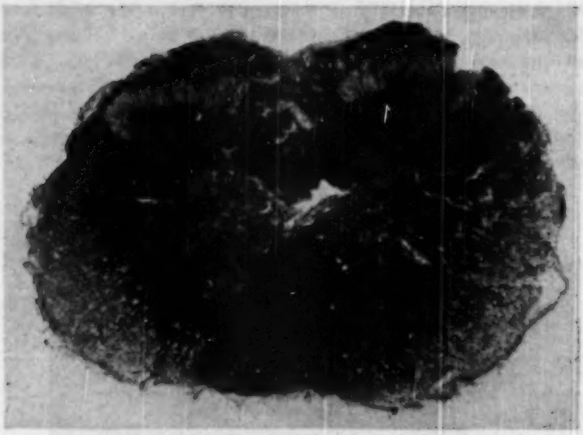

Fig. 7 (guinea pig 5).—Transverse section of the spinal cord showing extensive demyelination in the lateral and posterior columns. Spielmeyer stain; × 30.

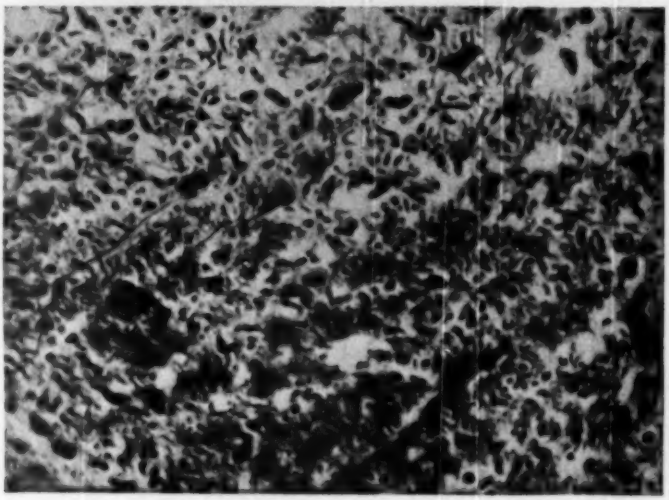

Fig. 8 (guinea pig 5).—Transverse section of the spinal cord in the midst of an extensive lesion, showing retention of many axons. Cajal's silver stain; × 400.

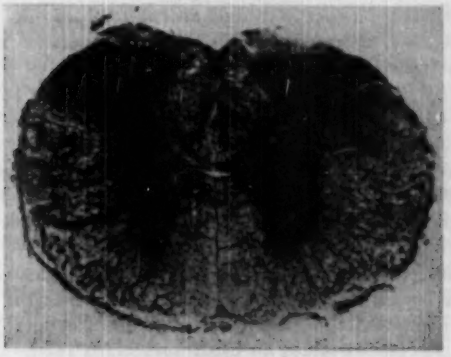

Fig. 9 (guinea pig 5).—Transverse section of the spinal cord showing vascular hyperplasia in the lateral and posterior columns. Foot's stain for reticulum; × 30.

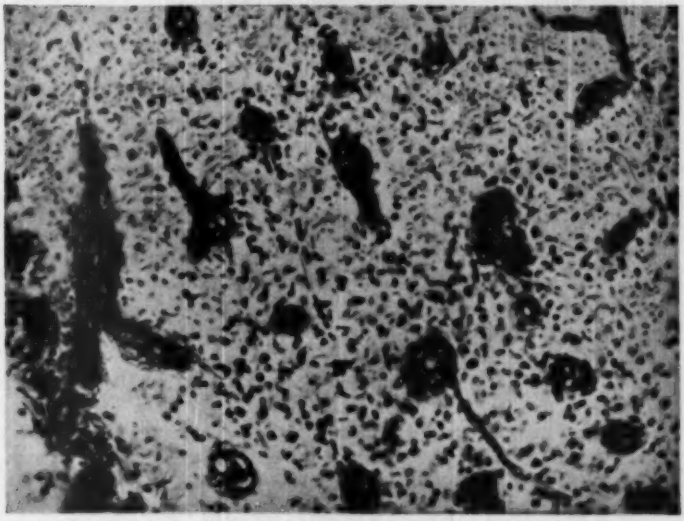

Fig. 10 (guinea pig 5).—Blood vessels in an affected zone of the spinal cord showing hypertrophy of the vascular wall. Foot's reticulum stain. × 100.

level. In one thoracic level of guinea pig 5 there was peripheral demyelination extending practically entirely around the cord (fig. 7), and this was in the zone where in the Nissl stain proliferation of microglia had been so conspicuous.

The axons, as revealed by Cajal's silver stain (fig. 8), were conspicuously spared. In the affected areas, a small number of axons were swollen, a few were absent, with empty sheath spaces remaining, but most of the axons were of normal size and number. Some of them were crowded together, being presumably pushed closer to one another, owing to the loss of myelin and the overgrowth of blood vessels (see fig. 9).

The blood vessels were increased in number in the most affected zones, as seen with various stains but particularly with the Van Gieson stain and Foot's stain for reticulum. But the thickness of the blood vessel wall was not accounted for by the presence of collagen, for the red coloration in Van Gieson stain was not sufficiently extensive. Foot's silver stain for reticulum showed an increased amount of that substance occurring in delicate, wavy meshes and greatly increasing the thickness of blood vessel walls (fig. 10). Often among the interstices of the reticulin in the vessel wall nuclei of various cells were present. Some of these nuclei were undoubtedly adventitial nuclei of the blood vessel wall in various stages of differentiation, while others were reacting hematogenous cells.

Gliosis was extremely scanty. In Holzer's stain thick strands of wavy fibrillar material, presumably reticulin, were mingled with a few astrocytes close to the blood vessels. But in Hortega's silver pyridine stain for astrocytes practically no gliosis was found.

COMMENT

The chronic nature of the lesions observed in these guinea pigs—four to six months—gives them characteristics not found in lesions of a more acute type. But the peculiarities of these lesions cannot be explained on the basis of chronicity alone. When the lesions are compared with the chronic lesions that were seen in rabbits as previously reported,[13] there are several distinct points of departure that may be mentioned. No fat was present in the oil red O or black sudan stains even though demyelination was distinct and in some instances extensive. It may be that fat was present in the early weeks of these lesions and had been more or less completely phagocytosed in the meantime. This would suggest that the process had come to an end and that demyelination was no longer taking place. On the other hand, rabbits with a survival time of four months presented brilliant red fat in the sudan stains as if the lesion were constantly expanding.

13. Morrison, L. R.: Arch. Neurol. & Psychiat. **58**:391, 1947.

Signs of gliosis were very scanty indeed in these guinea pigs, yet distinct early gliosis was reported in rabbits with a comparable survival time. Could this absence of gliosis have been related to the retention of axons? In the guinea pigs, in which gliosis was virtually absent, few of the axons were missing or even swollen, while in the rabbits previously referred to, axons were absent where gliosis was present.

The hypertrophy and hyperplasia of blood vessels together with the dense proliferation of reticulum in relation to them were a noteworthy feature in these chronic guinea pig lesions. Hyperplasia of reticulum is not characteristic of human multiple sclerosis, nor was it a feature in the rabbit experiments referred to, but in the guinea pigs, particularly in the spinal cord lesions, dense meshes of argyrophilic fibers surrounded the blood vessels from the subarachnoid space inward. The reticulum represented more than a hypertrophy of the wall of the blood vessels, and the hyperplasia of adventitial cells may have been the source of some of the macrophages present in the parenchymatous reaction.

The dearth of fat and the absence of gliosis indicate that the response is not identical in guinea pigs and rabbits. Whether this is more than a species difference remains as yet undetermined. The antigen employed in this experiment gives rise in some instances to a slowly developing lesion with a long survival time without tract degeneration, and it may be that the mildly advancing process does not stimulate the reaction of astrocytes. The retention of axons helps to explain why, in one of the animals at least, signs referable to the central nervous system later subsided.

The possibility cannot be excluded that the use of killed Myco. tuberculosis or Myco. butyricum grown on mediums containing glycerin and showing the usual growth characteristics would not induce the chronic form of disease, particularly if smaller amounts of bacilli or central nervous tissue were used.

SUMMARY

Mycobacterium butyricum grown on agar without glycerin differs from that grown on mediums containing glycerin with respect to morphologic and staining characteristics and spatial arrangements. This variant of Myco. butyricum was incorporated in a water-in-oil emulsion of guinea pig brain and injected subcutaneously into guinea pigs. Chronic encephalomyelitis developed in 4 and acute encephalomyelitis in 13 of 21 animals. In the chronic lesions there was rather extensive demyelination but no loss of axons and no gliosis.

A COMPARISON OF ARTERIOSCLEROTIC LESIONS PRODUCED IN YOUNG AND IN OLD RABBITS BY FREEZING THE AORTA

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THE REACTION evoked by injury and the reparative changes which follow the production of sclerotic lesions in senescent arteries have never been studied. Although arteriosclerotic lesions have been produced repeatedly in rabbits by use of epinephrine, ergosterol, diphtheria toxin and other substances, no attempt has been made to analyze the relation between age of animals and the development of experimental arteriosclerosis.

That senescent arterial tissues react to injury in a manner different from that of juvenile tissues might be predicted. Josué [1] described hard, brittle aneurysmal lesions occurring after repeated intravenous injections of epinephrine in rabbits weighing more than 2 Kg. Erb [2] repeated the work of Josué, and the most severe lesions described by him occurred in rabbits weighing more than 4 Kg. The age of the rabbits was not mentioned, and the method he used for the production of lesions was uncontrolled. The experiments reported here were designed to reproduce identical sclerotic lesions of the aorta in old rabbits and to evaluate the role of degeneration and regeneration in the arteriosclerotic process. The results are compared with those previously obtained in a similar study of juvenile rabbits.[3]

METHODS

Male rabbits weighing between 3.2 and 4.2 Kg. were used. They ranged in age from 150 to 248 weeks at the time of the completion of the experiment. Each rabbit was anesthetized with ether. The abdominal aorta was dissected and frozen

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1. Josué, M. O.: Compt. rend. Soc. de biol. **55**:1374, 1903.
2. Erb, W.: Arch. f. exper. Path. u. Pharmakol. **53**:173, 1905.
3. Taylor, C. B.; Baldwin, D., and Hass, G. M.: Arch. Path. **49**:623, 1950.

by the technic described in a previous publication.[3] Each rabbit was allowed to live from one to twenty-four weeks and then was killed by an intravenous injection of thiopental sodium (pentothal sodium®). The aorta was removed and fixed in Zenker's solution. Local lesions were resected, embedded in paraffin and cut transversely in serial sections. Sections were stained with hematoxylin and eosin and, when desired, with Weigert's stain for elastic tissue and Mallory's phosphotungstic acid-hematoxylin stain for connective tissue. Control sections were taken from the proximal and distal ends of the aortic segment containing the lesion. The section of the lesion showing maximum change was then selected for description and appraisal. All sections were graded according to the criteria used in the previous investigation of arteriosclerosis of juvenile rabbits.[3]

Lesions were produced in 10 senescent rabbits, which were allowed to live for intervals of time ranging from one to twenty-four weeks. Serial sections of 50 lesions were prepared for microscopic study.

RESULTS

The tissue reaction at the site of the lesion produced by freezing manifested a changing pattern as time passed. It was possible to divide this reaction, as in previous studies of juvenile rabbits, into several distinct processes which could be followed throughout the experimental period of six months. To facilitate the analysis, all processes which changed the structure of tissues further and further from the normal were arbitrarily defined as degenerative. These culminated in severe sclerotic disease similar to that described in man by Mönckeberg.[4] Processes of prolonged duration which tended to reproduce normal structure and which culminated in partial repair of the lesion were arbitrarily defined as regenerative.

Degeneration of the aorta proceeded as follows: Immediately following freezing, an aneurysmal dilatation of the aorta occurred at the site of injury. This was the result of death of smooth muscle cells within the media. Disintegration of cells was practically complete at the end of two weeks (fig. 1). Degeneration of elastic lamellas seemed to follow disintegration of smooth muscle. Individual elastic fibers of the media lost their normal wavy contour and became straight and fragmented. The internal elastic membrane appeared to remain undamaged. The external elastic membrane also retained its wavy form but frequently was incorporated as a discontinuous membrane in the fibrous tissue of the thickened adventitia. Calcification of the media was found in 23 of the 50 lesions. This appeared irregularly and was not significant in lesions less than 4 weeks old. After the loss of smooth muscle and the disintegration of the elastic structures had occurred, small deeply stained granules of calcium salts began to appear along the margins of the elastic lamellas. They increased in number until the interlamellar matrix became diffusely calcified. Calcification did not appear to be a prerequisite for development of bone and cartilage, nor was it permanent.

4. Mönckeberg, J. G.: Virchows Arch. f. path. Anat. **171**:141, 1903.

Among the 50 lesions 12 showed new formation of bone or cartilage. In no animal were both bone and cartilage found either in the same or in separate lesions. The appearance of bone or cartilage was unpredictable. The earliest trace of cartilage was found in lesions 4 weeks old (fig. 2). The earliest trace of bone was found in lesions 8 weeks old (fig. 3). Bone and cartilage formed principally in the adventitia and were present in the media only when the external elastic membrane was discontinuous. Fibroblasts seemed to develop into chondrocytes as a matrix of hyaline cartilage appeared around them (fig. 2). Bone appeared in a similar fashion. Osteoblasts differentiated from fibroblasts and became sur-

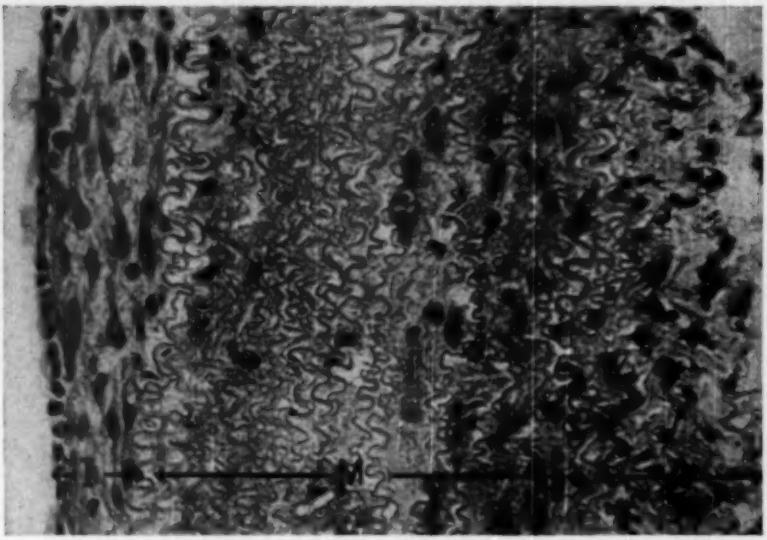

Fig. 1.—This is a low power photomicrograph of a transverse section of the aortic wall at the site of a lesion 2 weeks old. The letters *I*, *M* and *A* with arrows indicate the limits, respectively, of the intima, the media and the adventitia. Early stages of intimal proliferation with fibroblasts and immature smooth muscle cells are shown. The original structure of smooth muscle of the media has already disintegrated and disappeared. Although there is ingrowth of fibroblasts as well as differentiation of new smooth muscle cells in the periphery of the media, these are developing between the persistent remnants of fairly well preserved wavy elastic lamellas. There is mild fibrosis of the adventitia. Inflammatory reaction is negligible.

rounded by osteoid matrix (fig. 3). Calcification followed, and at times marrow developed. Usually, the entire fragment of bone was outside the external elastic membrane, and the media showed no sign of calcification. At times, the marrow showed hemopoietic activity (fig. 3).

Fig. 2.—This is a low power photomicrograph of a transverse section of the aortic wall at the site of a lesion 4 weeks old. The letters *I*, *M* and *A* with arrows indicate the limits, respectively, of the intima, the media and the adventitia. The intima is composed largely of condensed fibroelastic tissue with scattered fibrocytes and new smooth muscle cells. The media is thin, atrophic and fibrous. The adventitia is occupied largely by a plaque of cartilage, which is in various stages of formation and differentiation. Calcification is not apparent here.

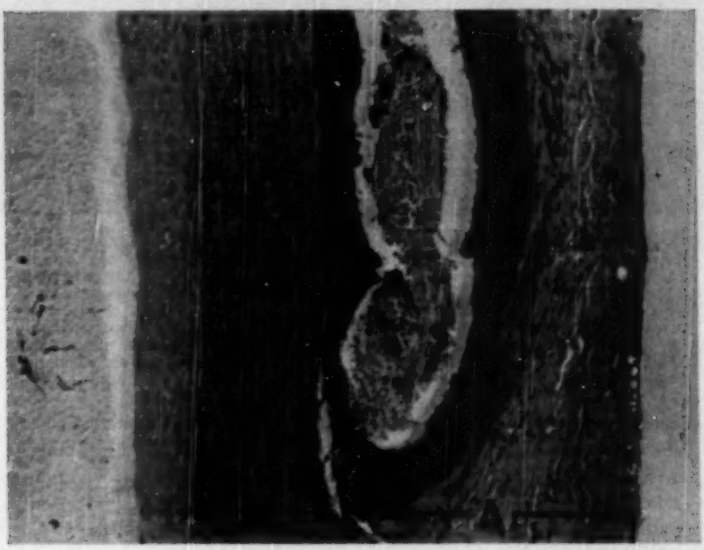

Fig. 3.—This is a low power photomicrograph of a transverse section of the aortic wall at the site of a lesion 8 weeks old. The letters *I*, *M* and *A* with arrows indicate the limits respectively of the intima, media and adventitia. The thickened intima is composed principally of fibroelastic tissue and smooth muscle. The media, except for sparseness of cells and loss of wavy contour of elastic lamellas, is fairly well preserved at this point. The adventitia contains a plaque of bone with well formed marrow spaces.

Periaortic inflammation was relatively inconspicuous in senile rabbits, but most sections showed fibrosis with fusion of collagenous fibrils in the adventitia. The character and the evolution of the degenerative changes are illustrated in a semiquantitative fashion in table 1.

In summary, among the processes classed as degenerative, calcification of the media was more variable and sporadic in senile than in young vessels. Formation of cartilage and bone was far more common in senile than in juvenile vessels and was not specifically related to medial calcifi-

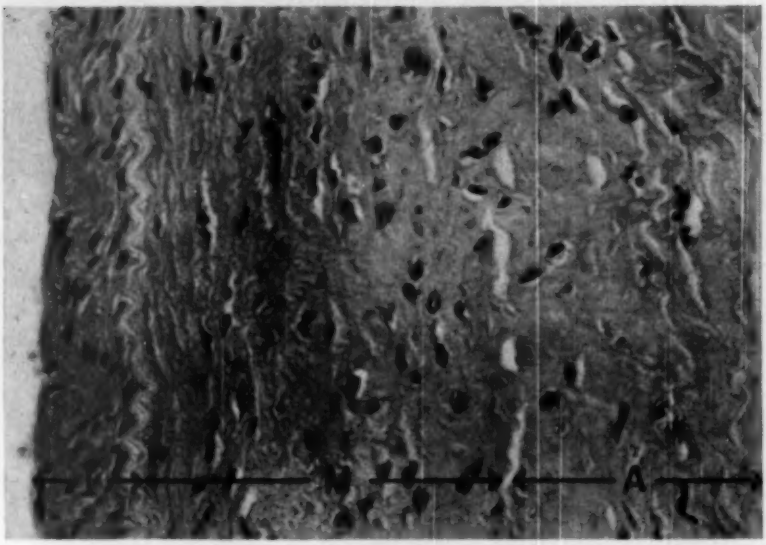

Fig. 4.—This is a low power photomicrograph of a transverse section of the aortic wall at the site of a lesion 12 weeks old. The letters *I*, *M* and *A* with arrows indicate the limits, respectively, of the intima, the media and the adventitia. The newly formed intima is composed largely of dense fibroelastic tissue with a few scattered fibrocytes and smooth muscle cells. The wavy internal elastic membrane is well preserved. The original structure of the media has been replaced by dense fibrous tissue incorporating remnants of elastic lamellas. Hyalinized fibrous tissue in the periphery of the media is continuous through spaces in the external elastic membrane with similar fibrous tissue in the adventitia.

cation, age of senile animals or the degree of fibrosis and inflammation of the adventitia. The process of degeneration of the senile aorta was otherwise practically identical with that of the juvenile aorta. Control sections taken from the aorta above and below lesions showed no spontaneous pathologic change, except for slight mucinous degeneration of the media of one senile aorta.

Regeneration began as early as the second week. Among the processes classified as regenerative, the proliferation of the senile intima was conspicuously inadequate as compared with the reaction of the juvenile vessel, described in a previous publication.[3] Although intimal proliferation had begun at 2 weeks in old rabbits, it never progressed to the advanced stage found in juvenile rabbits (fig. 1). Lesions as old as 12 weeks showed on cross section only a thin intimal strip proliferated around part of the circumference of the lumen (fig. 4). The strip of newly formed intima was never thicker than one-half the thickness of the normal aortic wall. Hence, it provided an inadequate matrix for regener-

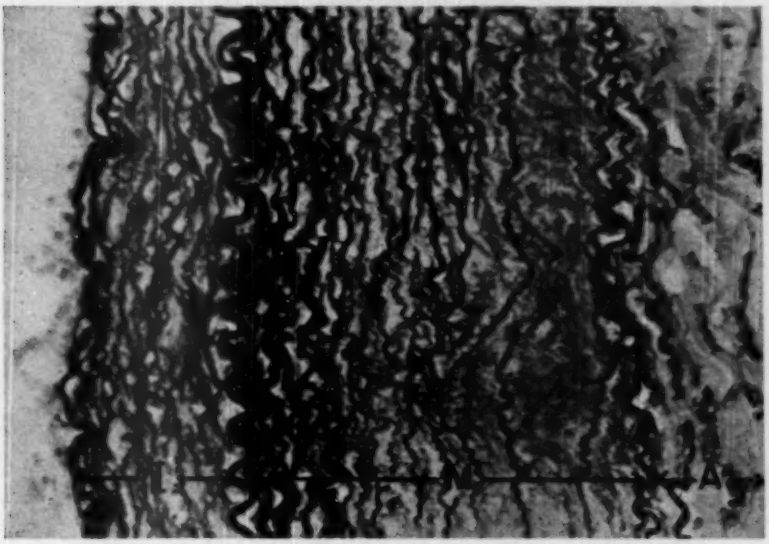

Fig. 5.—This is a low power photomicrograph of a transverse section of the aortic wall at the site of a lesion 20 weeks old. This has been stained by Weigert's method for demonstrating elastic tissue. The letters *I*, *M* and *A* indicate the limits, respectively, of the intima, the media and the adventitia. The newly formed intima has a new internal elastic membrane and a network of new elastic tissue. The original internal elastic membrane persists as a dense wavy membrane. External to this, in the media, most elastic lamellas are atrophic and discontinuous or absent. The persistent external elastic membrane and scattered fibrils in the adventitia are also shown.

ation of the constituents of the wall of the senile aorta. Aneurysmal sacs at sites of lesions were only partly filled in by proliferation of the intima, and local saccular aneurysms were still grossly apparent in lesions as old as 24 weeks. In juvenile rabbits the local aneurysms were promptly obliterated by the reparative processes.

Within the framework of the newly formed intima, smooth muscle cells developed and matured (figs. 1, 2, 3 and 4). Also, elastic lamellas developed within the proliferating intima in the same manner as in juvenile rabbits, and a new internal elastic membrane was regularly formed (fig. 5). Regeneration of smooth muscle within the damaged

TABLE 1.—*The Incidence of Degenerative Changes at Sites of Hypothermal Lesions of Different Ages in Aortas of Senile Rabbits*

Exper. No.	Age in Weeks: Aortic Lesion	Age in Weeks: Rabbit	Degeneration of: Smooth Muscle in Media	Degeneration of: Elastic Lamellas in Media	Deposits of Calcium in Media	Inflammation in Adventitia	Fibrosis in Adventitia	Lesions with: Cartilage	Lesions with: Bone
8	2	150	++	++	0	+	++	0	0
11	2.5	159	+++	+++	0	0	++	0	0
6	4	158	+++	+++	++	0	++	3	0
12	6	166	++	++	0	+	+++	0	0
3	8	244	+++	++	+	0	++	0	1
17	10	168	++	+++	++	0	++	0	0
10	12	172	++	++	0	0	++	1	0
4	17.5	226	++	++	0	0	++	0	0
9	20	188	++	+++	+	0	++	5	0
1	24	248	+++	+++	+	0	++	0	2

TABLE 2.—*A Summary of the Average Grades of Regenerative Changes in Aortic Lesions of Increasing Age in Old Rabbits.**

Exper. No.	Age in Weeks: Lesion	Age in Weeks: Rabbit	Proliferation of Intima	Proliferation of Elastic Tissue in Intima	Proliferation of Smooth Muscle in Intima	New Internal Elastic Membrane in Intima
8	2	150	1.6	0.8	1.5	0
11	2.5	159	2.0	1.0	0.8	0
6	4	158	2.3	2.0	2.7	1.0
12	6	166	2.3	2.0	2.4	2.8
3	8	244	2.9	2.4	2.6	2.6
17	10	168	3.0	3.2	2.5	3.3
10	12	172	2.3	3.5	2.7	4.0
4	17.5	226	2.0	3.0	3.0	4.0
9	20	188	2.9	3.0	3.0	3.3
1	24	248	2.4	3.0	2.0	2.4

* Similar grading of the lesions observed in young rabbits is shown in table 2 of a previous report.[3]

media was only slight. There was evidence of dissolution of the calcified areas within the media. This was manifested by a gradual loss of definition of the calcified plaques and by lacunar excavation of calcium deposits by giant cells.

A semiquantitative description of the activity of repair was made by using the criteria presented in a previous investigation of the aorta of the juvenile rabbit. In each rabbit the grades of change of the 6 lesions were averaged for each reparative process, and the values are given in

table 2. Intimal proliferation and regeneration of smooth muscle were active fairly soon after freezing of the aorta. Intimal proliferation never developed to the extent found in vessels of juvenile rabbits, although smooth muscle formation was practically complete within the framework

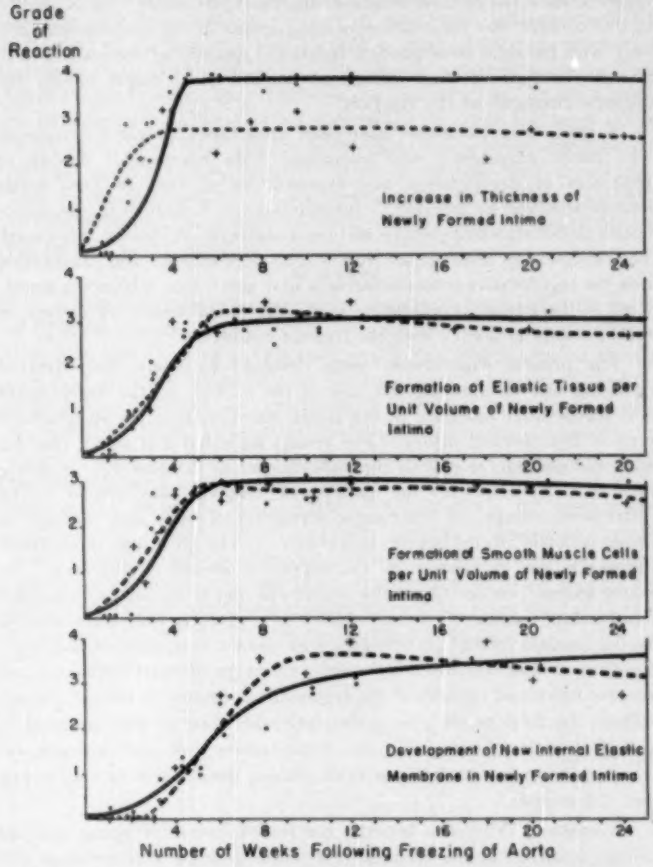
Grade of Reaction
Increase in Thickness of Newly Formed Intima
Formation of Elastic Tissue per Unit Volume of Newly Formed Intima
Formation of Smooth Muscle Cells per Unit Volume of Newly Formed Intima
Development of New Internal Elastic Membrane in Newly Formed Intima
Number of Weeks Following Freezing of Aorta

Fig. 6.—Comparison of curves of regenerative processes occurring in young rabbits, 3 to 9 months old (— .) and old rabbits, 38 to 61 months old (- - -+).

created by intimal proliferation. Regeneration of elastic tissue was a slower process, and formation of a distinct new internal elastic membrane was the most delayed. A comparison of these intimal regenerative changes in young and old animals is shown in figure 6.

COMMENT

The concept that the arteriosclerotic lesion is basically a local reaction of components of the vascular wall provoked by influences which upset the balance between functional demand and functional capacity of the vessel to fulfil the demand has been discussed previously.[2] It is implied in this concept that the reaction of components of the vascular wall will vary with the local structure and functional capacity of the wall, as well as with local or systemic extramural factors which might modify the ultimate character of the reaction.

A series of experiments have been undertaken to test this concept. The initial experiment was concerned with microscopic studies of sequences of degeneration and regeneration at sites of local aortic lesions produced by freezing.[3] Juvenile rabbits, 6 months of age, maintained under standard dietary and environmental conditions, were used. The conspicuous result other than medial degeneration and calcification was the regenerative construction of a new aortic wall within the framework of the intima, proliferating at the site of the lesion. This type of response was in accord with the concept outlined.

The present experiments were designed to study the effect of changing one variable, namely, age of the animal, on the degenerative and regenerative reactions of the aortic wall following the standardized type of hypothermal injury. The results indicated that senile vascular walls did not react in exactly the same manner as vascular walls of young animals. Nevertheless, the pattern of reaction was similar. The differences, except for heterotopic formation of bone and cartilage in senile animals, were largely quantitative. The principal quantitative difference was in relation to the degree of intimal proliferation. In young animals proliferation of the intima was very prominent and afforded an abundant framework within which a new vascular wall differentiated. In old animals intimal proliferation was limited in magnitude and often abortive, so that limitations were imposed on the ultimate perfection and relative functional capacity of the regenerated segment of vessel. Nevertheless, the final result was qualitatively the same as that obtained in young animals. Intimal fibrosis, hyalinization and atheromatosis, so conspicuous in human senile arterial disease, were absent in both young and old animals.

A frequent difference between the healed lesions of young and old rabbits occurred in the form of heterotopic formation of cartilage and bone. This was common in old animals but was encountered only rarely in the young. The formation of cartilage and bone occurred primarily in the adventitia of the degenerate aortic wall. No animal showed formation of both cartilage and bone. Even when there were multiple lesions in an animal, the reaction in separate lesions was always toward the for-

mation of either cartilage or bone. The explanation for this reactive disposition in the adventitial connective tissue of senile animals is not clear. It is in accord with similar reactions in arterial walls and elsewhere in aged human beings. It is commonly believed that in man the reaction is related to the age of the lesion. The experiments indicate that the reaction is to be correlated with age of the animal and not specifically with that of the lesion. The possible bearing of this observation on osteogenesis in other locations in aged people, as about joints in senile hypertrophic arthritis, may deserve some consideration. In view of the medial calcification commonly encountered in the aortic lesions of rabbits at all ages, an attempt was made to determine whether deposition of calcium was a necessary precursor of chondrogenesis and osteogenesis. This relationship could not be established, quantitatively or spatially. In fact, deposition of calcium was less frequent and less conspicuous in old than in young rabbits.

SUMMARY

Local hypothermal arteriosclerotic lesions were produced in the aortas of 10 senile rabbits. The sequences of degeneration and regeneration of the aortic wall were studied microscopically over a period of six months. The findings were compared with those obtained in a previous study of aortic lesions produced in young rabbits. Two principal differences were encountered. First, the senescent rabbits showed limited proliferation of intima at sites of lesions, so that the regeneration of a new aortic wall was quantitatively less than that encountered within the framework of the proliferating intima in young rabbits. Qualitatively the regenerative reactions were similar. Second, the aortic adventitial connective tissue of senile rabbits frequently showed cartilage or bone formation. This heteroplastic reaction was only rarely encountered in young animals and seemed to bear no established relation to anything other than the age of the animal in which the hypothermal lesion was produced.

Case Reports

BASAL CELL CARCINOMA OF THE LABIUM MINUS PUDENDI

BRUCE H. SMITH, M.D.*
BOSTON
AND
WILLIAM T. DAILY, M.D.
BROOKLYN

BASAL CELL carcinoma of the vulva is uncommon. Wilson [1] reported that it had been encountered 4 times in 66,000 surgical specimens at Duke Hospital, in Durham, N. C. His review of both American and foreign literature reveals only 23 reported cases. Berman [2] reported a case involving the labium minus, but the lesion was too far advanced when first seen to ascertain whether it arose on the labium minus or the labium majus, since both were involved. Our case, we believe, is the first reported case of primary involvement of the labium minus, to which the lesion was limited.

REPORT OF A CASE

Our patient is a 56 year old white woman born in the Netherlands. Her menstrual history was not unusual, with menarche at the age of 16 and a four to five day flow every twenty-six days. She had undergone two normal pregnancies and has two sons, aged 27 and 21 years. There were no abortions. Eleven years ago she noticed a small, painful area on her right labium minus which burned when urine flowed over it. Three years ago she passed the menopause but still had occasional hot flushes at the time she presented herself. The lesion on the labium showed exacerbations and remissions until several months before admission when another small, rounded, raised lesion appeared at the base of the labium minus near the forchette. This area was especially tender, and after consulting one of us (W. T. D.) she was admitted to Long Island College Hospital for its removal.

Physical examination on admission showed the right labium minus to be slightly enlarged, and the median surface appeared denuded. Another apparently denuded small area was found on the posteroinferior surface near the forchette corresponding to the nodule described by the patient. The remainder of the pelvic and the general examination showed essentially normal conditions. No enlarged lymph nodes were palpated in the groins. On Nov. 16, 1948, the patient was taken to the operating room and the right labium minus was removed under general anesthesia. Two days after the operation the patient had a mild pneumonitis, for which she was treated with 300,000 units of penicillin (duracillin®) daily for three days. Her postoperative course was thereafter uneventful, and she was discharged to her home on the eleventh postoperative day.

From Long Island College Hospital, Brooklyn.

* Now at New England Deaconess Hospital Laboratory of Pathology, Boston.

1. Wilson, V. M.: Arch. Surg. **43**:101-112, 1941.

2. Berman, W.: Am. J. Obst. & Gynec. **42**:1070-1076, 1941.

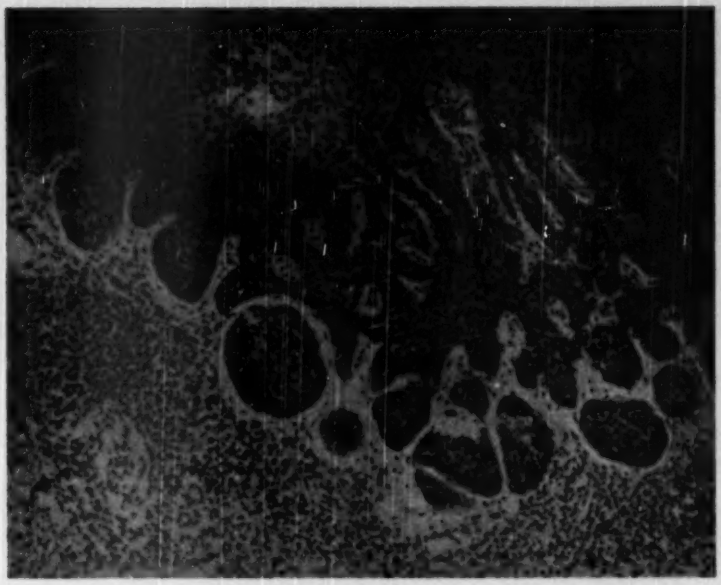

Fig. 1.—Neoplasm showing club-shaped invasive formations.

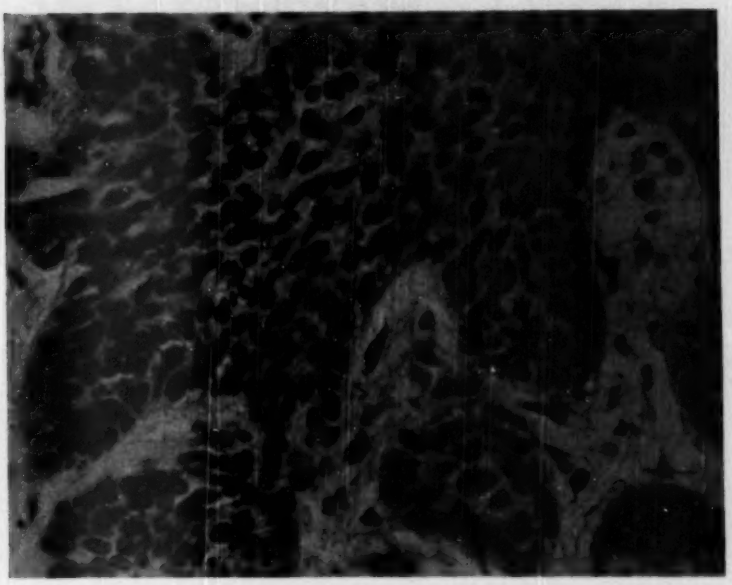

Fig. 2.—High power view of an area of the neoplasm shown in figure 1.

The specimen received by the department of pathology was a flexible crescentic piece of tissue, measuring 3.5 by 0.6 by 0.3 cm. Externally the tissue presented one somewhat roughened, dull surface, while the remaining surfaces, were smooth and glistening. Microscopically, this labium minus showed transformation of a wide portion of one epithelial surface into a cancerous neoplasm which somewhat superficially but definitely invaded the subepithelial tissue. This neoplasm was composed of small polygonal to spindle shape cells of the basal epithelial cell type, presenting hyperchromatic basophilic nuclei and scanty, eosinophilic cytoplasm. The cells were arranged in irregular club-shaped downgrowths, which presented a pseudocolumnar appearance at their periphery. Mitotic divisions were focally numerous. The stroma was moderately fibrous in nature and showed subepithelial and perialveolar infiltrations of lymphoid cells, macrophages and occasional eosinophilic cells of the granulocytic series (figs. 1 and 2). The interesting feature of this lesion was the relatively wide area involved with only superficial invasion. It is obvious from a histologic standpoint that this neoplasm did not date back the eleven years noted in the patient's history but more nearly coincided with the several months' history of recent exacerbation. There is no histologic evidence of what the predisposing lesion was; it is too early to predict the outcome of the case.

Since Wilson's [1] review of the literature (including the reports by Claiborn and Holsinger [3] and Schreiner and Wehr [4]) 4 more cases of basal cell carcinoma of the vulva have been reported by Berman,[2] and this case brings the total to 28, being the first case of basal cell carcinoma arising on the labium minus.

The photomicrographs were made by Dr. Richard G. McManus, resident pathologist of Long Island College Hospital, Brooklyn.

3. Claiborn, L. N., and Holsinger, H. B.: Surg., Gynec. & Obst. **54**:836-838, 1932.

4. Schreiner, B. F., and Wehr, W. H.: Surg., Gynec. & Obst. **58**:1021-1026, 1934.

Notes and News

Deaths.—Dr. Francis Harbitz, until 1938 professor of pathologic anatomy at the University of Oslo, died at the age of 83. He had taught pathologic anatomy to many generations of students and had been prominent as a medicolegal expert. Among his most important works were studies of endocarditis and of tuberculosis of childhood. He was at one time chairman of the Norwegian National Association Against Tuberculosis and the Cancer Committee of the Norwegian Medical Association and had been active in many other scientific organizations in Norway, Sweden, Denmark and Finland.

William Dean Collier, Youngstown, Ohio, formerly professor and director of the department of pathology of the St. Louis University School of Medicine, died March 15, aged 53, of cerebral thrombosis, arteriosclerosis and hypertension. He was a specialist certified by the American Board of Pathology, a member of the American Association of Pathologists and Bacteriologists and the American Society of Clinical Pathologists, and was a fellow of the Association for the Advancement of Science.

George William Schelm of Battle Creek, Mich., died March 11, aged 59, of carcinoma of the liver. A specialist certified by the American Board of Pathology and a founding fellow of the College of American Pathologists, he served on staffs of various Veterans Administration hospitals and was formerly affiliated with the Thomas D. Dee Memorial Hospital in Ogden, Utah. At the time of his death he was on the staff of the Leila Y. Post Montgomery Hospital.

Appointment.—Dr. Virgil H. Moon, emeritus professor of pathology in Jefferson Medical College of Philadelphia, has been appointed professor of pathology at the University of Miami, Fla.

American Society for Experimental Pathology.—At the meeting of the American Society of Experimental Pathology on April 21 in Atlantic City, N.J., James F. Rinehart, M.D., San Francisco, was elected president; F. S. Robscheit-Robbins, Ph.D., Rochester, N. Y., vice president; Sidney C. Madden, M.D., Uptown, N. Y., secretary-treasurer, and D. Murray Angevine, M.D., Madison, Wis., and Russell L. Holman, M.D., New Orleans, councilors.

Books Received

GRUNDRISS DER ALLERGIE—THEORIE UND PRAXIS. By Dr. Med. Rudolf Abderhalden. Pp. 179, with 6 illustrations. Price 14.50 francs. Benno Schwabe & Co., Klosterberg 27, Basel 10; imported by Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1950.

Abderhalden has written his monograph on theory and practice of allergy for rapid orientation of general practitioners and medical students. Thus he has covered representative trends of allergy in a small volume. The size of the book accounts for some shortcomings which are common to most condensed outlines of complex and enlarging subjects. Of these shortcomings, two are outstanding. First, the simplified presentation of a controversial concept tends to mislead the unsuspecting reader into hasty conclusions. While the reviewer agrees, for instance, with the author's contention that allergic diseases are diseases of civilization, he does not feel that the author has proved his point. Second, the limited space forces the author to emphasize certain aspects of allergy at the expense of others. For example, the book deals almost entirely with the anaphylactic type of allergy, neglecting the bacterial and eczematous types. Another example: The discussion of eosinophils, which this reviewer regards as a major problem in allergy, occupies less than ten lines.

On the other hand, the book is distinguished by a well selected bibliography, which includes work done on both sides of the Atlantic: this could serve as an example, since previous monographs in the field used to restrict bibliographic references to papers written in their own languages.

MAX SAMTER, M.D.

WATER AND SALT DEPLETION. By H. L. Marriott, C.B.E., M.D., F.R.C.P., Middlesex Hospital, London, England. Pp. 80, with 10 illustrations and 6 tables. Price $2. Charles C Thomas, 301-327 E. Lawrence Ave., Springfield, Ill., 1950.

METHODS IN MEDICAL RESEARCH, VOLUME 2. By Julius H. Comroe Jr., editor in chief. Pp. 361, with numerous illustrations and tables. Price $6.50. The Year Book Publishers, Inc., 200 East Illinois Street, Chicago, 1950.

THE 1949 YEAR BOOK OF PATHOLOGY AND CLINICAL PATHOLOGY (JANUARY-DECEMBER 1949). Pathology edited by Howard T. Karsner, M.D., LL.D., medical research advisor, Bureau of Medicine and Surgery, United States Navy; former professor of pathology, Western Reserve University, Cleveland. Clinical pathology edited by Arthur Hawley Sanford, M.D., professor of clinical pathology, University of Minnesota (Mayo Foundation); emeritus consultant, Division of Clinical Laboratories, Mayo Clinic. Pp. 543, with 161 illustrations. Price $4.75. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1950.

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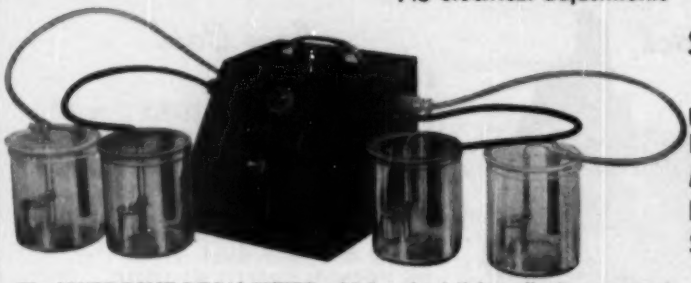

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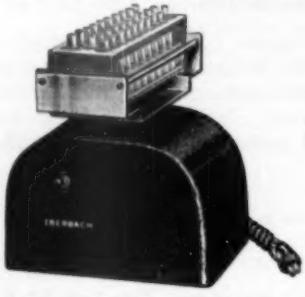

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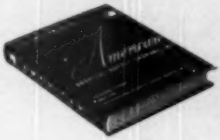

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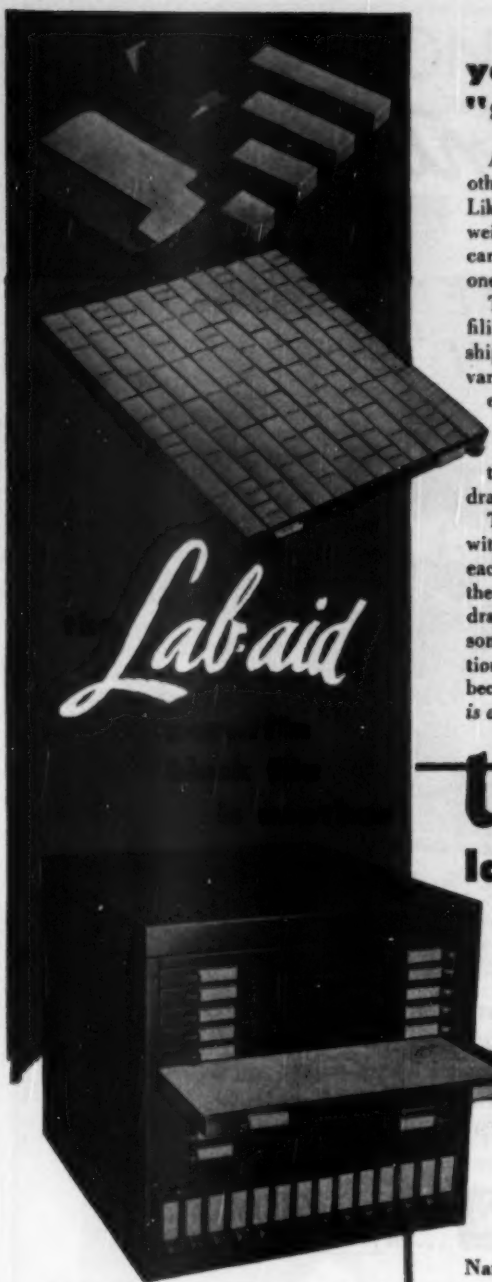

The "Lab-aid" paraffin block filing cabinet is finished to match all other types of "Lab-aid" files, and stacks interchangeably with any of them. Here it is stacked upon a vertical microslide file with 1" drawers.

you needn't put up with "shoebox" block filing

Ask for a six-month-old paraffin block in many an otherwise well-ordered laboratory, and the hunt's on. Likely as not it means hours of prowling through a weird behind-the-scenes jumble of dusty, dog-eared cartons . . . trying everyone's temper, taking everyone's time, piling up hidden costs.

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The all-steel cabinet holds ten drawers, equipped with combination drawer-pulls and label-holders at each end. Each drawer will accommodate a gross of the 1½" boxes: other sizes in ratio (see the filled drawers above). Multiply that by ten and you've some idea of the large capacity of this compact section (it's only 19" square by 12" high). That's because there's no waste space . . . *every cubic inch is a filing inch.*

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